frontiers Abstract Book

IMMUNO MEXICO 2018

XII Congress of the Latin American Association of Immunology and
XXIII Congress of the Mexican Society of Immunology

ISBN: 978-2-88945-511-9
DOI: 10.3389/978-2-88945-511-9

May 14-18, 2018, Cancún, Quintana Roo, México

Welcome to Immuno Mexico 2018, XII Congress of the Latin American Association of Immunology and XXIII Congress of the Mexican Society of Immunology!

The Latin American Association of Immunology (ALAI) was founded in 1984 in Buenos Aires, Argentina, and was accepted as a Federation by the International Union of Societies of Immunology (IUIS) in 1987. Our primary goals include the improvement of education in Immunology, training, and collaboration in experimental and clinical research in human and veterinary Immunology, and the triennial organization of the Latin American Congress of Immunology. The membership of ALAI embraces near 1,600 Latin American immunologists from Argentina, Brazil, Chile, Mexico, Colombia, Venezuela, Uruguay, Paraguay, Cuba, Peru, and Bolivia. Immunologists in Latin America have long shown substantial experience in the study of chronic infectious diseases. Thus, this 2018, ALAI academic activities feature the XII Latin American Congress of Immunology, in pivotal collaboration with the Mexican Society of Immunology, which co-celebrates its XXIII National Congress. Our scientific program provides many examples of fundamental knowledge and the mechanisms underlying immunity in health and disease, and with the most recent advances or emerging research in basic, clinical and translational Immunology. This book of abstracts includes original research divided into 18 topics including infectious, neoplastic and metabolic diseases, as well as allergy, immunodeficiencies, autoimmune or other chronic-degenerative disorders, conventional and novel bridge cell populations, technological and theoretical approaches for chronic disease complex systems and immunotherapy. We believe this book of abstracts show a small sample of recent research performed in the region and the remarkable development of Immunology in Latin America.

LIST OF ORGANIZERS

Latin American Association of Immunology - ALAI
Mexican Society of Immunology - SMI

Table of Contents

Immunology of Infectious Diseases

T cell immunology

701 The antagonic effects of the tumor necrosis factor and the brain derived neurotrophic factor on cholinergic neuritogenesis and viability

704 Understanding the autophagy-inflammation correlation in the Alzheimer´s disease development

707 An enriched environment restores glucose homeostasis, reduces inflammation and modifies microRNA levels in the hypothalamus of obese mice

710 IFN-γ induces a tolerogenic phenotype on macrophages/ microglia during the chronic phase of experimental autoimmune encephalomyelitis

711 Participation of the hypothalamic TRPV1 receptor in the reproductive axis

713 Effect of 17β-estradiol on oxidative stress and antioxidant activity in female mice infected with P. berghei ANKA

715 Acetone fraction from the edible roots of Sechium edule (Jacq.) S.w. possess anti endothelial dysfunction activity

718 Mycobacterium tuberculosis induces neuroinflammation and behavioral abnormalities during progressive pulmonary tuberculosis

720 Contribution of vasopressin to pulmonary tuberculosis immunopathology

722 Vitamin D, Melatonin, Psychoneuro-endocrine-immunology and aging in Curitiba, Brazil

727 Effect of DHEA in diabetes type 2 & tuberculosis comorbidity

728 Effect of Caspase-1-dependent inflammation on the protein levels of the BDNF/TRKB and TNF/TNFRII neuroprotector modules in a familial Alzheimer´s disease mouse model

Immune Regulation

857 Mucosal exposure to cigarette components induces intestinal inflammation, colitis and Paneth cells alterations in mice

858 Analysis of intestinal lymphoid populations in BALB/c mice underwent moderate exercise

861 The diminution of the alergic sensitization by a probiotic and a synbiotic is vinculated to changes in the intestinal mucosa in asthmatic rats

863 Lack of Macrophage Migration Inhitory Factor (MIF) Attenuates inflammation and clinical signs of experimental dry eye disease

864 Polysaccharides as target of thymus-independent mucosal immune responses and possible cross protection

868 Interleukin 6 activates STAT3 in colonic intestinal epitelial cells

869 STAT signaling in Intestinal Epithelial Cells during colitis

871 Effect of cholecystokinin (CCK) on the production of IgA in the small intestine of mice

873 IFN-gamma regulates STAT3 activation IL-6 mediated in epithelial cells during colitis

875 Effects of stimulation of muscarinic receptors in the secretion of IgAs intestinal in mice

876 High fat diet feeding modifies experimental dry eye disease in a strain-dependent manner

878 Antibody responses against *Saccharomyces cerevisiae*, D-mannose and D-glucose in Crohn's disease patients and non-ill subjects

883 Effect of Muscarine and Atropine on production of myeloperoxidase by neutrophils in lamina propia of small intestine

Vaccines

Veterinary Immunology

1003 Antibodies generated against bovine CD205 using a bioinformatics approach

1004 Differential responses to a Bovine respiratory disease challenge based on Bovine Herpes Virus 1 antibody titer status

1006 Retrospective analysis of relationships between pro-inflammatory, hematology, and metabolic variables, and mortality following a bovine respiratory disease challenge in weaned calves

1008 Characterization of cDC1 and cDC2 and the expression of DEC205 on porcine lymphoid tissues

1009 Tilmicosin a key regulator of inflamation in bovine mammary gland

1011 Production and development of an ELISA-PPA for the diagnosis of Mycobacterium avium subsp. paratuberculosis in red deer (Cervus elaphus)

1013 Development of an indirect elisa based on conserved peptides for the detection of antibodies against Babesia bigemina

1015 Macrophage apoptosis induction by proteins and lipids of Mycobacterium bovis

1016 Isolation and identification of turkey pox virus and development of an homologous vaccine prototype

1018 Development of a multiplex test for diagnosis of goat diseases

1022 Atypical granuloma formation by Mycobacterium bovis in young calves

1048 Multiepitope VLPs of B19 parvovirus reduce tumor growth and lung metastasis in aggressive breast cancer model

1051 Mesenchymal stem cells derived from human bone marrow, dental pulp, gingival tissue and periodontal ligament: In vitro evaluation of their differentiation and immunosuppressive capacities

1054 Arthritogenic peptides presented by tolerogenic dendritic cells are able to reprogram effector CD4+ T cell responses from rheumatoid arthritis patients

1057 Potential therapeutic application of newcastle disease virus for the treatment of lymphoma

1058 Arthritogenic peptides presented by dendritic cells re-establish tolerance

1061 Effects of Mesenchymal Stem Cells on endothelial dysfunction induced by Shiga toxin, the causative agent of Hemolytic Uremic Syndrome

1063 The unfolded protein response sensor IRE-1α modulates innate recognition and antigen presentation of melanoma tumor cells

1065 Improvement of antitumor immune response by adding a HIF-1a inhibitor in the immunization against melanoma in mice

1068 Effect of human intravenous immunoglobulin on the expression of Fc gamma receptors in monocytes and granulocytes

1070 Cell-permeable Bak BH3 peptide expressed on the surface of Salmonella enterica induce chemosensitization of tumor cells

1072 Monoclonal antibodies specifically targeting protein β-sheet secondary structure can diminish toxic oligomers and pathology in neurodegenerative diseases

1101 Interesting diversity of full HLA class I and class II genes typed using NGS (Next Generation Sequencing) in the Mestizo population from the city of Oaxaca, South-East Mexico

1103 Polymorphism analysis of 24 Short Tandem Repeats (STRs) in a large sample of healthy mexican mestizo subjects studied for paternity testing

1105 ALTHEA Gold Libraries™: novel antibody libraries for therapeutic antibody discovery

FOCIS

1106 Cortactin is highly expressed in B-cell precursor cells of childhood acute lymphoblastic leukemia: Implications for transendothelial migration and relapse

1110 Production, selection and characterization of monoclonal antibodies against outer membrane vesicles derived from Bordetella pertussis

1113 Il-33 and the pro-inflammatory response of colonic fibroblasts from patients with inflammatory bowel disease are modulated by interleukin-17

1115 Immunomodulatory effects of a third generation synthetic nitroalkene in solid organ allotransplantation

1117 Siglec-1 promotes the activation of human neutrophils

1118 Role of CD43 in the establishment of the immune microenvironment during Mycobacterium tuberculosis infection

1120 Promising anti-inflammatory and antimicrobial peptide isolated from the mexican tree frog Pachymedusa dacnicolor in the treatment of inflammatory skin diseases

1122 Molecular and genetic description of primary immunodeficiencies in mexican pediatric patients

1125 Molecular mechanisms of ubiquitin-mediated NETosis regulation in patients with systemic lupus erythematosus

1128 SEB stimulation induces functional pathogenic features in Th17 cells from psoriasis patients

1130 A prospective study unveils innate and adaptive immunity abnormalities as key risk factors for infections in patients with systemic lupus erythematosus: Results from the Germen cohort

1133 Bone marrow organoid-like reveals functional hierarchy governed by the niche in B-cell leukemia-initiating cells

1136 IL1β negatively regulates antiviral immunity in a mouse model of viral hepatitis

1139 Tmem176b is a checkpoint in IL-1β-dependent tumor immunity

1140 Characterization of the Th2 environment and local IgE production in colorectal polyps of allergic patients

1142 In vitro alloantigen specific Tr1 differentiation and expansion for therapeutic use in kidney transplantion

1144 Generation of large numbers of functional and stable human allo-specific induced-Tregs in the presence of pro-inflammatory cytokines

1146 CRTAM+ NK cells endowed with suppressor properties arise in leukemic bone marrow

1149 Immunoscoring in bone marrow aspirates as a novel tool for prognosis in acute leukemia patients

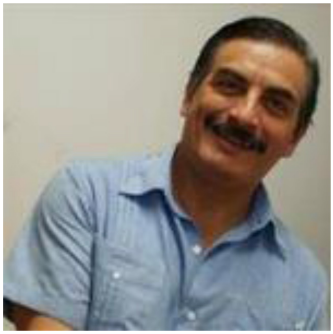
1152 The Vm24 scorpion toxin blocks Kv1.3 potassium channels and attenuates the effector memory T cells response

Translational Immunology

Board of Directors ALAI & SMI

Leopoldo Santos-Argumedo
ALAI President
Molecular Biomedicine Department,
Center for Research and Advanced Studies of the National Polytechnic Institute,
Mexico.

Rosana Pelayo
SMI President
Oncology Research Unit & Eastern Biomedical Research Center.
Mexican Institute of Social Security,
Mexico.



Humberto Lanz
ALAI Secretary
Center for Research on Infectious Diseases,
National Institute of Public Health,
Mexico.

Gustavo Pedraza
SMI Secretary & Treasurer
Department of Molecular Medicine and Bioprocesses,
Institute of Biotechnology,
National Autonomous University of Mexico, Mexico.

XII Congress of the Latin American Association of Immunology – ALAI
XXIII Congress of the Mexican Society of Immunology - SMI

MAY 14-18, 2018
CANCUN, QUINTANA ROO, MEXICO

WELCOME MESSAGE

On behalf of the Latin American Association of Immunology (ALAI) and the Mexican Society of Immunology (SMI), it is a pleasure to invite and welcome you to the XII Congress of ALAI and the XXIII Congress of SMI in Cancun, Quintana Roo, Mexico.

ALAI was founded in 1984 in Buenos Aires, Argentina, and was accepted as a Federation by the International Union of Societies of Immunology (IUIS) in 1987. Our major goals include the improvement of education in Immunology, training and collaboration in experimental and clinical research in human and veterinary Immunology, and the triennial organization of the Latin American Congress of Immunology. The membership of ALAI embraces near 1,600 Latin American immunologists from Argentina, Brazil, Chile, Mexico, Colombia, Venezuela, Uruguay, Paraguay, Cuba, Peru and Bolivia.

This year's ALAI academic activities feature the XII Latin American Congress of Immunology in collaboration with the Mexican Society of Immunology, which co-celebrates its XXIII National Congress.

We believe that delegates to this Congress will not only find an attractive academic program of remarkable science, but also a multitude of opportunities to connect with immunologists representing all IUIS federations and to interact in a relaxed atmosphere with leaders in the different areas of immunology. The Scientific Program Committee has organized an outstanding schedule of lectures, symposia and workshops that will provide you with fundamental

knowledge and mechanisms underlying immunity in health and disease and with the most recent advances or emerging research in basic, clinical and translational Immunology. Additionally, you will find novel events around fellow scientists and young Latin American investigators, poster sessions and social activities.

In Cancun, the core of a precious Mayan archaeological area and part of the world's second-longest coral reef, you will enjoy the most beautiful beaches on the Caribbean Sea blended with history and rich pre-Columbian culture. Cancun is today the most important tourist destination in the country, with a highly connected airport and excellent facilities to conduct an event of this magnitude and to offer unbeatable attractions. Transparent sea water, white sand and a bright sun with the warmth of Mexican hospitality will frame our unique academic celebration.

Please join ALAI-SMI in Cancun, Mexico, from May 14th to May 18th, 2018, and maximize your experience participating in the pre-Congress Cytometry meeting on May 13th-14th.

Sincerely,

Leopoldo Santos-Argumedo
ALAI President, *Chair*

Rosana Pelayo
SMI President, *Co-Chair*

International Academic Committee

Leopoldo Santos-Argumedo, Mexico
ALAI President
Department for Molecular Biomedicine at the Center for Research and Advanced Studies of the National Polytechnic Institute, Mexico.
Scientific Field: B Lymphocyte Biology, Primary Immunodeficiencies.

Rosana Pelayo, Mexico
SMI President
Oncology Research Unit & Eastern Biomedical Research Center. Mexican Institute of Social Security, Mexico.
Scientific Field: Early Lymphopoiesis, Tumor Immunology, Acute Lymphoblastic Leukemia, Emergency Microenvironment.

Humberto Lanz, Mexico
ALAI Secretary
Center for Research on Infectious Diseases, National Institute of Public Health, Mexico.
Scientific Field: Insect Immunity, Evolutionary Immunology & Ecology.

Gustavo Pedraza, Mexico
SMI Secretary & Treasurer
Department of Molecular Medicine and Bioprocesses, Institute of Biotechnology, National Autonomous University of Mexico, Mexico.
Scientific Field: Neuroimmunobiology, Inflammation, miRNAs, Lung Cancer.

Lourdes Arriaga, Mexico
SMI Cytometry Chapter Representative
Medical Research Unit on Immunochemistry, National Medical Center "Siglo XXI", Mexican Institute for Social Security, Mexico.
Scientific Field: Flow Cytometry, Systemic Inflammatory Response Syndrome, Sepsis, Biomarkers.

Laura Bonifaz, Mexico
Medical Research Unit on Immunochemistry, National Medical Center "Siglo XXI", Mexican Institute for Social Security, Mexico.
Scientific Field: Dendritic Cell Biology, Antigen Presentation, Innate Lymphoid Cells, Psoriasis.

Michael Schnoor, Mexico
Department for Molecular Biomedicine at the Center for Research and Advanced Studies of the National Polytechnic Institute, Mexico.
Scientific Field: Leukocyte Transmigration, Cortactin and Actin Molecules, Endothelial and Epithelial Barrier, Inflammation.

Constantino López, Mexico
Medical Research Unit on Immunochemistry, National Medical Center "Siglo XXI", Mexican Institute for Social Security, Mexico.
Scientifics Field: Long-lasting Immunity, Vaccines, Immunomodulation, Salmonella pathobiology.

Alejandro Chabalgoity, Uruguay
Biotechnical Development Department in the Hygiene Institute, School of Medicine, University of Uruguay, Uruguay.
Scientific Field: Salmonella Infection, Inflammasome Modulation, Non-Hodgkin Lymphoma, Cancer Immunotherapy.

Marcelo Hill, Uruguay
Laboratory of Immunoregulation and Inflammation, Immunobiology Department, Institut Pasteur of Montevideo, Uruguay.
Scientific Field: Cellular Therapy, Antigen Presentation, Immunomodulation, Autoimmunity, Cancer Immunology.

Fabiola Osorio, Chile
Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Chile.
Scientific Field: Antigen Presentation, Dendritic Cells, Unfolded Protein Response, T-lymphocytes.

Álvaro Lladser, Chile
Laboratory of Gene immunotherapy at "Fundación Ciencia & Vida", Chile.
Scientific Field: Cancer Vaccines, Tumor-killer T cells, T-lymphocytes.

Guillermo Giambartolomei, Argentina
Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Argentina.
Scientific Field: Brucella Lipoproteins, Adjuvants, Vaccines.

Adriana Gruppi, Argentina
School of Chemical Sciences of the National University of Córdoba, Argentina.
Scientific Field: Trypanosoma cruzi infection, B Cell immunobiology, Autoimmunity.

María Regina D'imperio Lima, Brasil
Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, Brasil.
Scientific Field: Immunology, Tuberculosis, Malaria.

Karina R. Bortoluci, Brasil
Department of Biological Sciences Federal Universityt of São Paulo, Brasil.
Scientific Field: Innate immunity, Inflammasomes and Macrophages.

Carlos Villegas, Cuba
National Institute of Oncology and Radiobiology, Havana, Cuba.
Scientific Field: Cancer Immunology, Tumor Microenvironment, Immunolabelling.

Deyanira La Rosa, Cuba
Institute of Gastroenterology, Havana, Cuba.
Scientific Field: Clinical Immunology, Immunodeficiencies, Viral Infections.

Oliver Pérez, Cuba
Immunology Department at Institute of Preclinical and Basic Sciences (ICBP) "Victoria de Girón", University of Medical Sciences of Havana, Cuba.
Scientific Field: Vaccines, Mucosal Adjuvants, Immunomodulation.

Luis F. García, Colombia
Group of Cellular Immunology and Immunogenetics. School of Medicine, University of Antioquia, Medellín, Colombia.
Scientific Field: T Lymphocytes, Tuberculosis, Immunology of Infectious Diseases.

Cristina Alonso-Vega, Bolivia
School of Medicine, Universidad Mayor de San Simon Cochabamba, Bolivia.
Scientific Field: Inflammation, Congenital Infections, Innate Immunity, Immunology of infectious Diseases.

Patricia Rodríguez, Bolivia
School of Medicine, Universidad Mayor de San Simon Cochabamba, Bolivia.
Scientific Field: Congenital Infections, Innate Immunity, immunology of Infectious Diseases.

National Academic Committee

Rogelio Hernández-Pando, Mexico
SMI Vice-president
Experimental Pathology Section, Department of Pathology. National Institute of Medical Sciences and Nutrition “Salvador Zubiran”. Mexico.
Scientific Field: Experimental Immunopathology, Immunopathology of Tuberculosis, Mucosal Immunology.

Rommel Chacon-Salinas, Mexico
SMI Pro-Secretary
Immunology Department, National School of Biological Sciences. Instituto Politécnico Nacional, Mexico.
Scientific Field: Mast Cell Biology, Innate Immune Response, Bacterial infection.

Yvonne Rosenstein, Mexico
Mextirg Focis Center of Excellence Director
Institute of Biotechnology, National Autonomous University of Mexico. Morelos, Mexico.
Scientific Field: T Cell Biology, Immune Regulation, Immunotherapy.

Karina Chávez-Rueda, Mexico
Immunology Research Unit, Pediatrics Hospital. Mexican Institute for Social Security, Mexico.
Scientific Field: B Cell Biology, Endocrino-Immunology, Systemic Lupus Erythematosus.

José Luis Maravillas, Mexico
Research Support Network, Coordination of Scientific Research, National Autonomous University of Mexico, Mexico.
Scientific Field: B Cell Biology, Non-Hodgkin Lymphoma, Autoimmunity.

Eduardo Ferat Osorio, Mexico
SMI Clinical Chapter Representative.
Medical Research Unit on Immunochemistry, National Medical Center "Siglo XXI", Mexican Institute for Social Security, Mexico.
Scientific Field: Clinical Immunopathology, Inflammation, Systemic inflammatory Response Syndrome, Acute Pancreatitis and Gastrointestinal Disorders.

Iris Estrada, Mexico
Immunology Department, National School of Biological Sciences. Instituto Politécnico Nacional, Mexico.
Scientific Field: Immunopathology of Mycobacterium Tuberculosis, Exovesicles, Neutrophil Biology.

Gloria Soldevila, Mexico
Instituto de Investigaciones Biomédicas, National Autonomous University of Mexico, Mexico.
Scientific Field: Immuneregulation, T Cell Biology, Oncoimmunology, Transplant Immunology.

Ana Flisser, Mexico
Faculty of Medicine, National Autonomous University of Mexico, Mexico.
Scientific Field: Immunomodulation By Taeniid Helminths.

Leticia Cedillo-Barrón, Mexico
Department of Molecular Biomedicine, Center for Advanced Research, The National Polytechnic Institute, Cinvestav-IPN, México.
Scientific Field: Arbovirus, Dengue Innate Immune Response, Dengue Immunopathogenesis.

Jennifer Enciso, Mexico
Eastern Biomedical Research Center, Medical Institute for Social Security.
Centro de Ciencias de la Complejidad (C3), National Autonomous University of Mexico, México.
Scientific Field: B Cell Differentiation, Acute Lymphoblastic Leukemia, Leukemic Microenvironment, Systems Biology.

Full Academic and Social Program

Monday, May 14th

13:00-17:00 **Registration**

17:00-18:00 **Inaugural ceremony**

Welcome messages:
Dr. Alberto Mantovani. President of the International Union of Immunological Societies IUIS
Dr. Leopoldo Santos-Argumedo. President of the Latin American association of Immunology ALAI
Dr. Rosana Pelayo. President of the Mexican Society of Immunology SMI

Special guests:
Dr. Jules Hoffman. Nobel Laureate in Medicine 2011
Dr. Oliver Pérez. Vice-President of ALAI
Dr. Ethel García-Latorre. Former ALAI and SMI President
Dr. Sergio Estrada-Parra. Founder of the SMI

18:00-19:00 **Opening plenary lecture**

"Innate Immunity from Insects to Mammals"
Nobel Laureate Dr. Jules Hoffman. Strasbourg University Institute for Advanced Study, FRA.
Chair: Dr. Humberto Lanz. INSP, MEX.

19:00-21:00 **Cocktail reception**

Tuesday, May 15th

8:00-9:00 **Plenary lecture 1**

"The yin-yang of innate immunity, inflammation and cancer"
Dr. Alberto Mantovani. Humanitas University, ITA.
Chair: Dr. Eva Salinas. U.A.Ags, MEX.

9:00-10:00 **Plenary lecture 2**

"Shaping the immune landscape by galectin-driven regulatory circuits: Therapeutic opportunities in cancer, autoimmunity and infection"
Dr. Gabriel Rabinovich, IBYME, ARG.
Chair: Dr. Alejandro Escobar. InDRE, MEX

10:30-12:30 **Symposia 1-4**

Symposium 1: Innate and adaptive cell development

Dr. Marco Colonna, Washington University, USA.
Dr. Ellen Rothenberg, Caltech, USA.
Dr. Avinash Bhandoola, NCI, NIH, USA.
Dr. Juliana Idoyaga, Stanford University School of Medicine, USA.
Chair: Dr. Laura Bonifaz. IMSS, MEX.
Co-chair: Dr. Rosana Pelayo. IMSS, MEX.

Symposium 2: Mucosal immunology-lamig session

Dr. Sergio A. Lira, Icahn School of Medicine at Mount Sinai, USA.
Dr. Francisco J. Quintana, Harvard Medical School. Boston, USA.
Dr. Daniel Mucida, Rockefeller University. USA.
Dr. Juliana Cassataro, UNSAM-CONICET. ARG.
Chair: Dr. Fernando Chirdo, ARG.

Symposium 3: B Cells, germinal centers and beyond

Dr. Joseph Craft, Yale University. USA.
Dr. Virginia Rivero, CIBICI-CONICET UNC, ARG.
Dr. Pablo Engel, U. de Barcelona, ESP.
Dr. Romina Gamberale, IMEX-CONICET, ARG.
Chair: Dr. Adriana Gruppi, CIBICI-CONICET UNC, ARG.

Symposium 4: Veterinary and comparative immunology

Dr. Corwin Nelson, Animal Sciences Dept, U. Florida, USA.
Dr. Juan Mosqueda, Fac Ciencias Naturales, UAQ, MEX.
Dra. Ana Jar, Fac Ciencias Veterinarias, U. Buenos Aires, ARG.
Dr. Sergio Rodríguez, CENID PAVET, INIFAP, MEX.
Chair: Dr. Laura Cobos, Fac Med Vet, UNAM, MEX.
Co-chair: Dr. José Angel Gutiérrez-Pabello. Fac Med Vet, UNAM, MEX.

12:30-13:30 **Commercial showroom**

Thermofisher science lunch

Dr. Ramiro Diz

13:30-15:00 **Poster session**

P1-P162

15:00-16:30 **Workshops 1-6**

Workshop 1: Innate immunity I

Opening Lecture: Dr. Bruno Rivas. IMSS Zacatecas, MEX. Antimicrobial peptides for the treatment of tuberculosis: allies or foes
Chair: Dr. Rommel Chacón. IPN, MEX
-Leslie Chavez-Galan. TNFR1 modulates the recruitment of CD3+TCR$\alpha\beta$-and CD3+TCR$\alpha\beta$+ myeloid cells to the liver after BCG infection
-Zayda L. Piedra-Quintero. Myosin 1F promotes macrophage pro-inflammatory phenotype during colitis by controlling AKT/STAT signaling
-Tonathiu Rodriguez. Macrophage galactose-type lectin (MGL) receptor plays a critical role in the infection against Trypanosoma cruzi
-Daiana Celias. Cathepsin L3 from Fasciola hepatica induces IL-1β and IL-18 secretion in a NLRP3 dependent manner on dendritic cells

-Carolina I. Serrano García. IFN-gamma regulates STAT3 activation IL-6 mediated in epithelial cells during colitis

Workshop 2: Tumor immunology

Opening Lecture: Alexander Pedroza. UNAM, MEX. Immunoregulatory cells in breast and liver cancer.
Chair: Dr. Ezequiel Fuentes. HIMFG, MEX.
-Luis Enrique Huanosta-Murillo NLRP3 nuclear translocation regulates IL-4 expression in patients with cutaneous lymphoma in early stage
-Thalia Pacheco-Fernández Macrophage migration inhibitory factor is a positive modulator if macrophage-driven-immune response in colorectal tumors at early stages of cancer progression
-Alvaro Lladser Vaccination-induced skin-resident CD8+ T cells mediate and broaden protective immunity against melanoma
-Paola Maycotte Autophagy regulates the secretion of Macrophage Migration Inhibitory Factor (MIF) from Breast Cancer Cell Lines
-Alberto Chinney-Herrera Exosomes produced by metastatic breast cancer cells induce more angiogenesis than non-metastatic breast cancer cells in spheroid culture conditions

Workshop 3: Clinical and translational immunology I

Opening Lecture: Zaima Mazorra. Center Clin Mol Immunol, CUB. Targeting EGFR in cancer patients: passive and active therapy.
Chair: Florencia Rosetti. INCMNSZ, SSA, MEX.
-Leonor Huerta Hernández. Contribution of HIV patient serum antibodies that recognize lymphocyte antigens to the inhibition of the HIV-1 envelope-dependent membrane fusion
-Nidia C. Moreno Corona. LRBA Characterization in B Lymphocytes

-Glauben T. Landskron. Relationship of Interleukin 33 with metastasis-supporting events in colorectal cancer patients
-Iris K. Madera-Salcedo. Hypermethylation of PPP2R2B represents a novel mechanism by which chronic inflammation perpetuates itself
-Cristina N. Aguilar-Flores. Evaluation of plasticity features in pathogenic Th17 lymphocytes from the blood and skin of psoriasis patients

Workshop 4: Immunology of infectious diseases I

Opening Lecture: Susan Bueno. IMII, CHI. Immune evasion mechanisms used by Salmonella to cause systemic infections.
Chair: Gladys Fragoso. IIB, UNAM, MEX.
-Joyoti Basu. The role of microRNAs in regulating autophagy and the inflammatory response during Mycobacterium tuberculosis infection
-Luis Castillo. Human Neutrophil Response Against the Hyper-Epidemic Clone of Carbapenem Resistant Klebsiella pneumoniae ST258
-Bernardita Medel. The unfolded protein response sensor IRE-1α modulates innate recognition and antigen presentation of melanoma tumor cells
-Samira Muñoz Cruz. Identification of Giardia lamblia molecules that contribute to mast cell activation and cytokine secretion

Workshop 5: T Cell immunology

Opening Lecture: Patricia Portales. UASLP, MEX. Regulators of metabolism in T lymphocytes of adipose tissue.
Chair: Karina Chavez. IMSS, MEX.
-Noe Rodriguez-Rodriguez. PP2A B55ß limits the lifespan of self-reactive and pathogen-specific CD8 T cells through the pro-apoptotic molecule HRK.

-Miguel Olivas-Aguirre. Cannabidiol suppresses human leukemic T cells via breakdown of the mitochondrial function: the role of intracellular Ca2+
-Sandra Ortega-Francisco. Inhibins modulate T cell activation and T effector function differentiation
-Felipe Campos-de-Almeida. Epigenetic drug 5-Azacytidine enhances potential killing of CD8 T Cells
-Mauricio Campos-Mora. Role of Nrp1 in Foxp3+Treg-dependent suppression mechanism

Workshop 6: Neuroendocrinoimmunology

Opening Lecture: Rodrigo Pacheco. Neuroimmunology Foundation Science and Life, CHI. Dopamine-driven inflammation in the gut and the brain.
Chair: Martha Legorreta. UNAM, MEX.
-Sol Díaz de León-Guerrero. An Enriched Environment restores glucose homeostasis, reduces inflammation and modifies microRNA levels in the hypothalamus of obese mice
-Mario Zetter-Salmón. Contribution of vasopressin to pulmonary tuberculosis immunopathology
-Jesús Aguilar Castro. Effect of 17β-estradiol on oxidative stress and antioxidant activity in female mice infected with P. berghei ANKA
-Martha Pedraza-Escalona. Understanding the autophagy-inflammation correlation in the Alzheimer´s Disease development
-Jacqueline Lara Espinosa. Mycobacterium tuberculosis induces neuroinflammation and behavioral abnormalities during progressive pulmonary tuberculosis

17:00-19:00 **Symposia 5-8**

Symposium 5: Inflammation and disease

Dr. Guadalupe Sabio, Fundación CNIC Carlos III, ESP.
Dr. Gabriel Nuñez, University of Michigan, USA.

Dr. Alyssa Hasty, Vanderbilt University, TN, USA.
Dr. Andrea Cossarizza. University of Modena, ITA.
Chair: Dr. Gustavo Pedraza-Alva, IBT, UNAM, MEX.
Co-Chair: Dr. Leonor Pérez Martínez, IBT, UNAM, MEX.

Symposium 6: Women in immunology: networking

Dr. Olivera J. Finn, U. Pittsburgh, USA.
Dr. Clara Gorodezky. INDRE, MEX.
Chair: Dr. Yvonne Rosenstein, IBT UNAM, MEX.

IUIS Gender equality & career development Award Ceremony

Awarded: Dr. Clara Gorodezky

Symposium 7: The Interaction of Vector saliva pathogen: A big challenge for the host

Dr. Eric Calvo, NIH, USA.
Dr. Claudia Brodskyn, Fiocruz, BRA.
Dr. Berlin Londoño. Kansas State University, USA.
Dr. Fabiano Oliveira, NIH, USA.
Chair: Dr. Leticia Cedillo. CINVESTAV, MEX.

Symposium 8: Society for leukocyte biology: emerging topics in immune cell trafficking

Dr. Patricia Fulkerson, Cincinnati Children's Hospital Medical Center, USA.
Dr. Christoph Scheiermann, Ludwig-Maximilians-Universität München, GER.
Dr. Helen McGettrick, University of Birmingham, USA.
Dr. Caroline Jones, Virginia Tech, USA.
Chair: Lou Justement. University of Alabama at Birmingham, USA.
Co-Chair: Helen McGettrick, University of Birmingham, USA.

19:00-20:00 **Plenary lecture "Dr. Eduardo García Zepeda"**

"New Insights in Multiple Myeloma Clinical and Biomedical Research"
Dr. Rubén Niesvizky. Weill Cornell Medicine, USA.
Opening synopsis: Dr. Cynthia López. IIB UNAM, MEX.
Chair: Dr. Rosana Pelayo. IMSS, MEX
Co-chair: Dr. Leopoldo Santos-Argumedo. CINVESTAV. MEX.

Wednesday, May 16th

6:00-7:00 **Immunorace**

Dr. Mary Fafutis Morris. CUCS, UDG, MEX.

8:00-9:00 **Plenary lecture 3**

"Clinical and immunological consequences of reduced *Plasmodium falciparum* transmission"
Dr. Faith Osier. Kenya Medical Research Institute, KEN.
Chair: Dr. Ethel García-Latorre. ENCB, IPN, MEX.

9:00-10:00 **Plenary lecture 4**

"How B cells adapt in a changing world"
Dr. Susan Pierce. NIAID, NIH, USA.
Chair: Dr. Jesús Martínez-Barnetche. INSP, MEX.

10:30-12:30 **Symposia 9-12**

Symposium 9: Primary immunodeficiencies

Dr. Mohamed-Ridha Barbouche, Institut Pasteur de Tunis. TUN.
Dr. Menno C. van Zelm, Monash University, AUS
Dr. Francisco Espinosa-Rosales, National Institute of Pediatrics, MEX.
Dr. José Luis Franco, Universidad de Antioquia, COL.
Chair: Francisco Espinosa, National Institute of Pediatrics, MEX.

Symposium 10: Immune cell interactions in blood vessels

Dr. Paul Frenette, Albert Einstein College of Medicine, NY, USA.
Dr. Pilar Alcaide, Tufts Medical Center, Boston, USA.
Dr. Mirta Ana Schattner, IMEX, CONICET, Buenos Aires, ARG
Dr. Michael Schnoor, CINVESTAV-IPN, MEX.
Chair: Dr. Michael Schnoor, CINVESTAV-IPN, MEX.

Symposium 11: Viral immunology and adjuvants

Dr. Michael R. Parkhouse, Gulbenkian Institute, POR.
Dr. Fernando Esquivel Guadarrama. UAEM, MEX
Dr. Oliver Perez, SLD, CUB.
Dr. Susan Kovats. OMRF, OKC, USA.
Chair: Dr. Oliver Perez, SLD, CUB.

Symposium 12: Focis goes to ALAI 2018

Dr. Ignacio Anegón, FCE, FRA.
Dr. Flavio Salazar. FCE, IMII, CHI.
Dr. Martín Rumbo, FCE, IIFP, ARG.
Dr. Yvonne Rosenstein, MEXTIRG FCE, MEX.
Chair: Dr. Guillermo Docena, CONICET, ARG.

12:30-13:30 **Commercial showroom**

Science lunch

German research foundation: Research in germany

Maxi Neidhardt, DFG Office Latin America. German Research Landscape
Dr. Michael Schnoor, CINVESTAV, MEX
Dr. Christoph Scheiermann, Munich University, GER
Dr. Faith Osier, KEMRI-Wellcome Trust Research Programme (Kenya)/Heidelberg University Hospital (Germany)
Dr. Christina Siebe, DFG Liaison Scientist in Mexico
Susanne Faber, DAAD Office Mexico City

13:30-15:00 **Poster session**
P163-P324

15:00-16:30 **Workshops 7-12**

Workshop 7: Antibodies and B Cell biology

Opening Lecture: Dr. Jesus Martínez-Barnetche, INSP, MEX. Hidden insights in B cell biology revealed by repertoire and transcriptome data mining.

Chair: Dr. Juan Carlos Rodríguez, U Veracruzana, MEX.

-Eloísa Arana. Characterization of cytokine-producing B cell subsets in human tonsils, insights into their role in tonsillar hypertrophy and recurrent tonsillitis

-Daniel A. Giron Perez. Myosin Ie supports the migration of B cells

-Mireille A. Santamaria. Cellular and molecular characterization of human memory B cell response upon Dengue virus infection and other arboviruses

-Ernesto Acevedo-Ochoa. New insights of TSPAN33 function in B-cells

-Gustavo Caballero Flores. IgG, but not IgA, protects infant mice against oral C. rodentium infection

Workshop 8: Innate immunity II

Opening Lecture: Dr. Paula Licona. IFC-UNAM, MEX. PKIB and the PKA pathway controlling innate lymphoid cell differentiation and function.

Chair: Dr. Samira Muñoz. IMSS, MEX.

-Araceli S. Tepale-Segura. Cholera Toxin and its Non-Toxic β Subunit Promote the Recruitment and Training Features in Skin Dentritic Cells

-John F Arboleda-Alzate. TLR2-dependent sensing of dengue virus induces early RIPK1-mediated necroptosis and TNF-α production

-Ivan Lozada-Requena. Uncaria tomentosa is not cytotoxic neither apoptotic, increases ROS production and induce an M2 polarization in human monocytes-macrophages

-Marcia Campillo Navarro. Mast cells release extracellular traps against isoniazid-resistant Mycobacterium tuberculosis
-Clara Gorodezky. KIR3DL1, but not its Ligands, confers protection for the expression of Acute Lymphoblastic Leukemia (ALL) in Mexican Mestizo Patients

Workshop 9: Immunology of infectious diseases II

Opening Lecture: Marcela Henao. CSU, USA. Immune Mediated Pathogenesis in tuberculosis
Chair: Dr. Leonor Huerta. IIB-UNAM, MEX.
-Hernan F. Penaloza. IL-10 production by Myeloid derived suppressor cells during Klebsiella pneumoniae ST258 infection induces an anti-inflammatory environment required for host survival
-Natalia Taborda. Dysfunctional IL-17-producing CD8+ T cells are associated with high immune activation in HIV-infected patients under HAART-induced viral suppression
-Erick Sánchez Salguero. Increased amounts of IgA1 and IgA2 in human colostrum are associated with infections during pregnancy
-Emma Rey Jurado. Contribution of CD1d to immune responses and pathogenesis in mouse models of human Respiratory Syncytial Virus and the human Metapneumovirus infections
-Sandy Reyes Martínez. Role of SOCS3, SHP1-2 and PIAS3 proteins on the activation of STAT3 in macrophages during infection with Taenia crassiceps

Workshop 10: Immune regulation

Opening Lecture: Karina Pino-Lagos. University of Los Andes, CHI. Studiyng the immune response during allograft rejection with special emphasis on Tregs and other modulators.
Chair: Ricardo Lascurain. UNAM, MEX.

-Noe Rodriguez-Rodriguez. PD-1 regulates the expression of HELIOS and EGR2 in self-reactive CD8+ and $CD4^{-}CD8^{-}$ T cells
-Paulina García-González. Regulation of tolerogenic markers on human dendritic cells by MYC
-Marisol De La Fuente-Granada. Inhibins regulate peripheral induction of regulatory T cells through modulation of dendritic cells
-Teresa Freire. Novel Immunoregulatory Points to Control Fasciolosis
-Saúl Arteaga. Generation of large numbers of functional and stable human allo-specific induced-Tregs in the presence of pro-inflammatory cytokines

Workshop 11: Mucosal immunity and microbiome

Opening Lecture: Dr. Porfirio Nava. Epithelial barrier dysfunction during colitis: role of IFNgamma.
Chair: Dr. Leticia Moreno Fierros. UNAM, MEX.
-Luisa Cervantes-Barragan. Lactobacillus reuteri induces gut intraepithelial CD4+CD8 alpha alpha + T cells
-Marco Vega-Lopez. Intestinal immune response to nematodes in pigs
-Tomás Villaseñor. Exposure to an enriched environment attenuates mouse experimental colitis
- Angela Wahl. Two New In Vivo Model Systems to Directly Examine Human Gut Microbiota's Role in Human T Cell Development and Immunity
-Michael Reinhart. Exposure to all-trans retinoic acid (ATRA) during parenteral immunization increases mucosal immunity in an antigen-specific manner

Workshop 12: Systems immunology and immunoinformatics

Opening Lecture: Luis Mendoza. IIB-UNAM, MEX. A dynamical model of the regulatory network controlling lymphopoiesis.

Chair: Dr. Elisa Dominguez Huttinger. CCMatemáticas, UNAM, MEX.
-Jennifer Enciso. Modeling the multi-modular network of normal B lymphopoiesis blockage in acute leukemias
-Mariana Martinez-Sanchez. Regulatory Network Showcases Mechanisms Underling CD4+ T Cell Differentiation and Plasticity
-Helena Kuri-Magaña. Understanding Class Switch Recombination related non-coding (CSR-nc) transcription through experimental analysis and data mining of RNA-seq experiments at the NCBI Sequence Read Archive, the GTEx and TCGA projects
-Carlos Villarreal. Diabetes type 2 as an inflammatory disease: a complex network analysis
-Elisa Domínguez-Hüttinger. Mathematical modelling of complex host-pathogen interactions on epithelial tissues

17:00-19:00 **Symposia 13-16**

Symposium 13: Advances in innate immunity

Dr. Mihai Netea, Radboud Universiteit, HOL.
Dr. Elina Zuñiga, UCSD, USA.
Dr. Luis Cadavid, UNC. COL.
Dr. Loems Ziegler-Heitbrock. HZM, GER.
Chair: Dr. Humberto Lanz. INSP-SSa, MEX.

Symposium 14: Induction of long lasting immunity

Dr. Paul Klenerman, U. Oxford.GBR.
Dr. Gabriel Victora, The Rockefeller University. USA.
Dr. Burkhard Ludewig. KantonSSpital, St. Gallen, SUI.
Chair: Dr. Constantino López. UIMIQ, IMSS, MEX.

Symposium 15: Immunology of tuberculosis

Dr. Luis Fernando García, Universidad de Antioquia, COL.
Dr. Luciana Balboa, CONICET, ARG.
Dr. Marcela Henao, CSU, USA.
Dr. Mayra Silva Miranda, IIB-UNAM, MEX.
Chair: Dr. Iris Estrada. ENCB-IPN, MEX.

Symposium 16: Therapeutic antibodies

Dr. Juan C. Almagro. GlobalBio, Inc. Cambridge. USA.
Dr. Mary Ann Pohl. Tri-Institutional Therapeutics Discovery Institute, NY. USA.
Dr. Sonia Mayra Pérez Tapia. UDIMEB/UDIBI-ENCB, IPN, México.
Chair: Dr. Monica Guzman. Weill Cornell Medicine, USA.

19:00-20:30 **ALAI presidents meeting**

Thursday, May 17th

8:00-9:00 **Plenary lecture 5**

"Archaeological perspectives on maya civilization"
Dr. Rodrigo Liendo Stuardo. IIA, UNAM, MEX.
Chair: Dr. Susana del Toro Arreola. CUCS, UDG, MEX.

9:00-10:00 **Plenary lecture 6**

The power of ONE: Immunology in the age of single cell genomics
Dr. Ido Amit. Weizmann Institute of Science, ISR.
Chair: Dr. Juan Carlos Almagro. GlobalBio, Inc. Cambridge, USA.

10:30-12:30 **Symposia 17-20**

Symposium 17: Neuroimmunology

Dr. Hugo Besedovsky, Phillips University, GER.
Dr. Adriana del Rey, Phillips University, GER.
Dr. Edda Sciutto, IIB, UNAM, MEX.
Dr. Oscar Bottasso, IDICER-CONICET, ARG.
Chair: Dr. Rogelio Hernández-Pando. INNSZ- SSa, MEX.

Symposium 18: Immunoregulation

Dr. Alexander Rudensky, Memorial Sloan Kettering Cancer Center, USA.
Dr. Dan Littman, NYU, School of Medicine, USA.

Dr. Rosa Bacchetta, Stanford, CA, USA.
Dr. Marta Vives-Pi, IGTP, Badalona, ESP
Chair: Dr. Gloria Soldevila. IIB-UNAM, MEX.

Symposium 19: Immunity against bacteria

Dr. Dario Zamboni, USP Riverao Preto, BRA.
Dr. Paula Barrionuevo, IMEX-CONICET, ARG.
Dr. Susan Bueno, PUC, CHI.
Dr. Sergio C. Oliveira, UFMG, BRA
Chair: Dr. Antonio Enciso, IMSS, MEX.

Symposium 20: IUIS goes to ALAI 2018

Dr. Dietrich Kabelitz. UKSH, GER.
Dr. Gail A. Bishop. U. Iowa, USA.
Dr. Richard Goodman. U. Nebraska-Lincoln, USA
Chair: Dr. Humberto Lanz. INSP, MEX.

12:30-13:30 **Commercial showroom**
Solaria biodata science lunch

12:30-14:00 **Assembly and business meeting asociación latinoamericana de inmunología ALAI**

13:30-15:00 **Poster session**
P325-P484

15:00-16:30 **Workshops 13-18**

Workshop 13: Clinical and translational immunology II

Opening Lecture: Dr. Ignacio Terrazas. UNAM, MEX. Translational Medicine: Are we ready for helminth therapy?
Chair: Dr. Edith Oregon. UDG, MEX.
-Gabriela López Herrera. Mutations pattern of Primary Antibody Deficiencies by Next Generation Sequencing
-Marcelo Hill. Tmem176b is a checkpoint in IL-1β-dependent tumor immunity

-Flavio L. Matassoli. Hydroxypropyl-beta-cyclodextrin as a potential drug to control chronic inflammation in HIV infection by inhibiting the activation of myeloid cells by microbial products
-Ileana Torres Alarcón. Rheumatoid factor combined with Anti-Modified Citrullinated Vimentin is associated to risk cardiovascular in rheumatoid arthritis
-Laura Berrón-Ruiz. Low percentages of regulatory T cells in Common Variable immunodeficiency (CVID) patients with autoimmune diseases and association with increased numbers of CD4+CD45RO+ T and CD21low B cells

Workshop 14: Vaccines

Opening Lecture: Leandro Carreno. University of Chile, CHI. Optimizing NKT cell ligands as vaccine adjuvants.
Chair: Dr. Rosendo Luria. HIMFG, MEX.
-Luis A. Ontiveros-Padilla. Protection induced by intradermal immunization with Salmonella Typhi porins correlate with the induction of memory T cells and dendritic cell responses in skin and draining lymph node
-Alberto Juan Dorta Contreras. Neuroimmunological approach for evaluation of viral measles, mumps and rubella (MMR) triple vaccine
-Rosendo Luria. Cell-permeable Bak BH3 peptide expressed on the surface of Salmonella enterica induce chemosensitization of tumor cells
-Oliver Pérez. Alternatives to multiple doses of allergy´s immunotherapy

Workshop 15: Veterinary and comparative immunology

Opening Lecture: Dr. Monica Imarai. University Santiago de Chile, CHI. Salmon adaptive immune response.
Chair: Dr. Juan Mosqueda. UAQ, MEX.
-Diana Sánchez Castro. Potential Therapeutic Application of Newcastle Disease Virus for the treatment of Lymphoma

-Ismael Martínez Cortés. Tilmicosin a key regulator of inflamation in bovine mammary gland
-Lucinda Puebla-Clark. Characterization of cDC1 and cDC2 and the expression of DEC205 on porcine lymphoid tissues
-José Eduardo Nolasco Estrada. Isolation and identification of turkey pox virus and development of a homologous vaccine prototype

Workshop 16: Immunotherapy and immunotechnology

Opening Lecture: Dr. Tania Crombet. Center Mol Immunol, CUB. Lung cancer immunotherapy: The Center of Molecular Immunology approach.
Chair: Dr. Mayra Perez. ENCB, IPN, MEX.
-Yanelys Morera. Specific active immunotherapy with a VEGF targeted vaccine: from bench to bedside
-María Moreno. Attenuated Salmonella as neoadjuvant therapy for melanoma-bearing mice undergoing chemotherapy
-Fernando Goni. Monoclonal antibodies specifically targeting protein β-sheet secondary structure can diminish toxic oligomers and pathology in neurodegenerative diseases
-Olivier Boyer. Pathogenicity of auto-antibodies from man to mice: the case of immune-mediated necrotizing myopathies
-Juan Tellez Sosa. Development of human monoclonal antibodies against multidrug resistant bacteria

Workshop 17: Immunology of infectious diseases III

Opening Lecture: Dr. Pablo Gonzalez. Pontificia Universidad Católica de Chile, CHI. Identification of a herpes simplex virus type 2 mutant that enables dendritic cell functions that elicit protective antiviral immunity.
-Cuauhtemoc A. Sanchez Barrera. Taenia crassiceps infection induces the recruitment of low density neutrophils with suppressive activity

-Maria Menezes. IL-1α promotes liver inflammation and necrosis during blood-stage Plasmodium chabaudi malaria
-Ismael Mancilla-Herrera. Higher frequencies of activated naive CD4+CD32a+ T cells in newborn and infants from HIV+ mothers
-Nathaniel Schramm. Long-term persistence of Zika virus infection in the eye

Workshop 18: FOCIS

Opening Lecture: Dr. Mario Cruz. UAEM, MEX.
-Octavio Castro-Escamilla. SEB stimulation induces functional pathogenic features in Th17 cells from psoriasis patients
-Germán Galliussi. Immunomodulatory effects of a third generation synthetic nitroalkene in solid organ allotransplantation
-Juan C. Balandrán. Bone marrow organoid-like reveals functional hierarchy governed by the niche in B-cell leukemia-initiating cells
-Maite Duhalde Vega. IL1β negatively regulates antiviral immunity in a mouse model of viral hepatitis
-Estefanía Alemán Navarro. Role of CD43 in the establishment of the immune microenvironment during Mycobacterium tuberculosis infection

17:00-19:00 **Symposia 21-23**

Symposium 21: Clinical and translational immunology

Dr. Romina Goldszmid, NIH, USA.
Dr. Juan R. Cubillos-Ruiz, Weill Cornell Medicine, NYC, USA.
Dr. Monica L. Guzman, Weill Cornell Medicine, USA.
Chair: Dr. Arturo Cérbulo, UNAM, MEX.

Symposium 22: Immunity to parasites

Dr. Claudia Cristina Motran, Universidad Nacional de Córdoba, ARG.

Dr. Eva V Acosta Rodríguez, CIBICI-CONICET, ARG.
Dr. Edda Sciutto, IIB, UNAM. MEX.
Dr. Ana Flisser, Fac. Med. UNAM, MEX.
Chair: Dr. Ana Flisser, Fac. Med. UNAM, MEX.

Symposium 23: Inflammasomes

Dr. Dr. Mohamed Lamkanfi, Ghent University, BEL.
Dr. Vianney Ortiz, CINVESTAV, MEX.
Dr. Karina Bortoluci, Universidade Federal de São Paulo, BRA.
Chair: Dr. Karina Bortoluci, UFSP, BRA

19:00-20:00 **Special session on career development**

Dr. Olivera J. Finn, U. Pittsburgh, USA.
Dr. Clive Gray, Institute of Infectious Disease and Molecular Medicine, South Africa.
Dr. Dietrich Kabelitz, UKSH Campus Kiel / CAU, Institute of Immunology GER.
Dr. Luis Fabila Castillo, ENCB, IPN, MEX.
Chair: M.Sc. Jennifer Enciso, IMSS & UNAM, MEX.
Co-chair: Dr. Luisa Cervantes. WUSM, USA.

20:00-21:00 **Assembly and business meeting sociedad mexicana de inmunología-SMI**

21:00-24:00 **Immunoparty**

Friday, May 18th

8:30-9:30 **Plenary lecture 7**

"Modulating the immunological synapse to enhance pathogen immunity and reduce detrimental inflammation"
Dr. Alexis Kalergis, FCE, IMII, CHI.
Chair: Dr. Gustavo Pedraza. IBT-UNAM, MEX.

9:30-10:30 **Closing plenary lecture**

"Identification of two new cytokines: IL-40 and IL-41".

Dr. Albert Zlotnik. University of California Irvine, USA.
Chair: Dr. José Luis Maravillas. UNAM, MEX

10:30-11:30 **Awards and closing ceremony**

ALAI travel awards
IUIS gender awards
SMI national fellowships
National Immunology award
Young Investigator award “Dr. Sergio Estrada Parra”
PhD student award “Dr. Sergio Estrada Parra”
Oral presentation awards
Poster presentation awards
Immunorace awards
Acknowledgements from SMI Board
Message from the president of ALAI
Message from the president of SMI

Pre-Congress - First Iberoamerican Flow Cytometry Meeting

Sunday, May 13th

7:00-8:00	**Registration**
8:00-8:10	**Welcome. Dr. Lourdes Arriaga-Pizano, Mexico. MSI & ISAC Member**
8:10-9:10	**Opening Lecture** "Next generation cytometry ¿Are still cells to discovered?" Dr. Alberto Orfao (Centro de Investigación del Cáncer, Universidad de Salamanca-España)
9:10-11:00	**Symposium 1: Quality Control: Standarization & Harmonization** Dr Rodolfo Patussi Correia. Hospital Israelita Albert Einstein Sao Paulo, Brazil. "Flow Cytometry in the Clinical Laboratory: Assurance of the Quality Process from the Sample to the Final Report". Dr. Ricardo Morilla. The Royal Marsden Hospital, United Kingdom. "Instrument harmonization for high quality and reproducible data in line with ISO 15189" Dr. Juan Flores Montero. Universidad de Salamanca, España. "EuroFlow standardization for current flow cytometry applications" Chair: Dr. Roxana del Río Guerra. Co-chair: MSc Jessica L. Prieto-Chávez
11:00-11:30	**Coffee Break & Commercial Runway**

11:30-12:50 **Symposium 2: Nanoparticle & other Non-conventional systems analysis by flow**

Dr. Alfonso Blanco-Fernández. University of Dublin. "Your limitation is your imagination... and some technical difficulties"

Dr. Daniel Scott-Algara. Pasteur Institute. "Detection of RNA by cytometry: from the bench to screening biomarkers?"

Chair: Dr Heriberto Prado García

12:50-14:00 **Round Table: "Revisiting Major Myths in Flow Cytometry"**

Dr. Andrea Cossarizza. University of Modena and Reggio Emilia School of Medicine/ISAC President.

Dr. Robert Balderas. BDB, R&D Director

Dr. Henning Ulrich. Instituto de Bioquimica, Universidade de São Paulo, Brasil

Chair: Dr. Julio Enrique Castañeda; Co-chair: Dr. Ismael Mancilla-Herrera

15:00-17:00 **Workshops 1 & 2**

Workshop 1. Monitoring Immune system: Research and clinical applications

Dr. Gloria Soldevila. LABNALCYT UNAM. "Expansion and conversion of allospecific regulatory T cells for immunotherapy in kidney transplantation"

Dr. Daniela Lens. Hospital de Clínicas, Montevideo-Uruguay. "Monitoring immune response in chronic infectious diseases"

Dr. Alvaro Luiz Bertho. Flow Cytometry Core Facility, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brasil. "Flow cytometry approaches to study the immunopathogenesis of protozoan diseases: from microvesicles to cells"

Chair: Dr. Martha Moreno Lafont Co-chair Jairo Ricardo Villanueva Toledo

Workshop 2. Monitoring Immune system: Research and clinical applications

Dr. Julia Almeida. Centro Investigación del Cancer, Universidad de Salamanca, España. "Identification of T and NK cells and their major functional subsets by flow cytometry: application in immune monitoring"
Dr. Monica Guzman. Weill Cornell Medicine, New York, USA. "Cytomic approaches to evaluate selective targeting of leukemia stem cells in mice and human"
Dr. Diana L. Bonilla. MD Anderson Cancer Center. "Biomarker Discovery in Cancer Immunotherapy by using Multiparametric Cytometry"
Chair: Beatriz Pérez Romano

17:00-17:30 **Networking & Commercial runway**

17:30-18:30 **Closing Lecture: "The future of translational cytomics"**

Dr. Alejandro Ruíz Argüelles. Laboratorios Medica Sur, México
Chair: Dr. Arturo Cérbulo Vázquez

Monday, May 14th

8:00-16:00 **Simultaneous Flow cytometry practical workshops**

I. Becton Dickinson Biociencias: Estandarización de paneles de 8 colores en Onco-hematología Enfermedad Mínima Residual (EMR) Professors: Dr. Alberto Orfao, Julia Almeida y Daniela Lens
II. Merck Millipore: "Flow Imaging: CAR-T cells, FISH Flow, CRISPR Flow" Professors: Claudia Emanuele, Brian Hall y Andres Saralegui

III. Beckman-Coulter: Nanoparticles, EVS & Exosomes analysis by Flow Professors: Dr. Alfonso Blanco y Dr. Alvaro Luiz Bertho
IV. ThermoFisher: PrimeFlow® RNA Assay Professors: ThermoFisher & LabNalcit Staff

Special Workshop 7:00 a 9:00 Data Management: "Brains on" Workshop (For those that need a little help for the interpretation of flow cytometry data) Professors: Dr. Lourdes Arriaga-Pizano & BSc. Mihay Gonzalez García

Expression of LOX-1 in circulating phagocytes of patients with sepsis

Lorena Hernández Torres[1,2], Rommel Chacon-Salinas[1], Eduardo Antonio Ferat[2], Luis Angel Flores-Mejia[2,3], Berenice González Flores[4], Armando Isibasi Araujo[2], Patricia Esther Miranda Cruz[2,5], Lourdes Andrea Arriaga Pizano[2]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Unidad de Investigación Medica en Inmunoquímica, Mexico City, Mexico
[3]Programa de Doctorado en Ciencias Biomédicas, UNAM, México, Mexico
[4]Servicio de Medicina Interna, UMAE Hospital de Especialidades, CMN SXXI, México, Mexico
[5]Departamento de Biología Celular (CINVESTAV), México, Mexico
lorenfq.02@hotmail.com; rommelchacons@yahoo.com.mx; eduardoferat@prodigy.net.mx; anjl_72@hotmail.com; bgonzalezflores@hotmail.com; isibasi@prodigy.net.mx; paty-kmila@hotmail.com; landapi@hotmail.com

Keywords: Inflammation, Myeloid Cells, Phagocytosis, Sepsis, LOX-1

Introduction: LOX-1 (Lectin-like Oxidized low-density lipoprotein receptor-1) is an integral glycoprotein of type II membrane that belongs to the family of type C lectins. It is the main receptor for oxidized lipoproteins (OxLDL) but it also could bind lipopolysaccharide, TNF-α, interleukin-1 (IL-1), interferon-gamma (IFN-γ) and C-reactive protein (CRP). LOX-1 is found mainly on the surface of endothelial cells, in which induces the expression of adhesion molecules such as E-selectin, P-selectin and V-CAM, which favors the extravasation of circulating leukocytes. LOX-1 as an inducer of endothelial dysfunction has been implicated, in addition to atherosclerosis, in other pathologies such as hypertension and diabetes, which correlates with the fact that stimuli related to cellular stress, such as hyperglycemia and free radicals, induce the expression of LOX-1. The binding of oxLDL to LOX-1 increases intracellular reactive oxygen species (ROS) such as superoxide anion (O2-) and hydrogen peroxide (H2O2), in human monocytes from atherosclerotic lesions.

There is little evidence that relates LOX-1 with systemic inflammation. Inhibition of LOX-1 in a model of rat endotoxemia reduces leukocyte adherence to intestinal microcirculation, and diminishes plasma levels of MCP-1. In mouse models (C57/BL6) "knockout" for LOX-1, the survival of mice subjected to polymicrobial sepsis increases. This diminished mortality was related to decrease in serum and pulmonary levels of both TNF-α and IL-6, and a greater capacity for neutrophil migration. However, they

also observed the diminution in bacterial elimination capacity, which suggests that LOX-1 may be involved in the neutrophil dysfunction observed in sepsis.

Aim: To determine the expression of LOX-1 in phagocytes of healthy volunteers and patients with sepsis and establish its relation with phagocytosis.

Materials and Methods: From healthy volunteers and patients with sepsis, 10 mL of venous blood collected in a tube without anticoagulant (5 mL) and a tube with lithium heparin as anticoagulant (5 mL) for the analysis of circulating leukocytes will be recovered by humeral puncture. Monocytes (CD45medCD14+) and neutrophils (CD45lowCD16+) were identified and characterized for LOX-1 expression by flow cytometry.

Phagocytosis was also evaluated by flow cytometry using pHrodo Bioparticles Phagocytosis Kit, pH Green version. We identified cells able to endocyte by calculating the percentage cells pH Green+ and the bacterial elimination capacity by fold increase MFI.

Results: 21 subjects have been recruited; 10 healthy volunteers and 11 patients diagnosed with sepsis. In patients with sepsis analyzed there is leukocytosis, with significant neutrophilia ($p < 0.005$). In turn, these neutrophils increased in patients with sepsis, decreased their expression of LOX-1 ($p < 0.05$). LOX-1+ neutrophils conserved showed higher bacterial elimination than LOX-1-.

Conclusions: In mature neutrophils from patients with sepsis phagocytosis impairment seem to be related to LOX-1 diminished membrane expression.

PMNs as “Trojan horse” vehicles for Brucella abortus persistence and dispersion in murine bone marrow

María Cristina Gutiérrez-Jiménez[1], Ricardo Mora-Cartín[1], Alejandro Alfaro-Alarcón[2], Edgardo Moreno-Robles[2], Elías Barquero-Calvo[1,2]

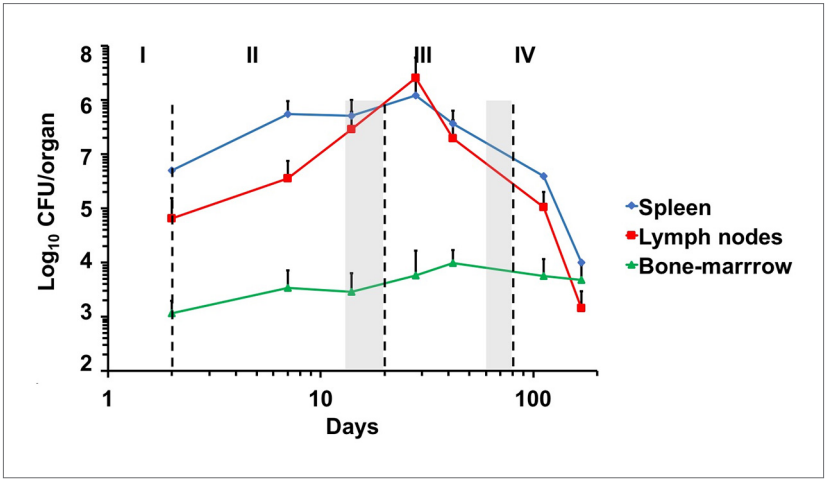
[1]University of Costa Rica, San José, Costa Rica
[2]National University of Costa Rica, Heredia, Costa Rica
christiarte@gmail.com; ricardomora1001@yahoo.com; aalfaroa@gmail.com; edgardo.moreno.robles@una.cr; elias.barquero.calvo@una.cr

Keywords: Bone Marrow, Brucella abortus, Cytokines, Macrophages, Neutrophils

Brucella abortus is a facultative intracellular pathogen that causes chronic infections. Neutrophils (PMNs) are the first cells that encounter Brucella after invasion, however, Brucella resist their killing action and induce premature cell death of these leukocytes 2,3,5. It has been described that B. abortus persist in bone marrow at chronic stages of infection 1. Nevertheless, the role of PMNs in bone marrow persistence has not been studied.

We show that B. abortus organisms are able to persist in murine bone marrow even at the “declining stages” of chronic infection. B abortus were observed inside a PMN/monocyte cell type at very low rates. Additionally, we demonstrate that murine bone marrow PMNs phagocyte antibody-opsonized B abortus and die quickly after infection. These dying infected PMNs show increased adhesion and are readily taken up by RAW 264.7 macrophages. When ex vivo macrophage infections were performed, B. abortus were more infective and replicated at higher rates when macrophages were infected through PMNs than when infected with Brucella only. In general, proinflammatory and anti-inflammatory cytokines showed increased values in macrophages infected through PMNs but only after 24 hours of infection, when Brucella has already reached their replication niche inside the cell.

Our results, support the notion that infected bone marrow PMN might behave as vectors for Brucella persistence in bone marrow in a non-logistic way.


I
II
III
IV
8
6
7
5
4
3
2
Log10 CFU/organ
1
10
100
Days
Spleen
Lymph nodes
Bone-marrrow

Expression of soluble and membrane IL-6 receptor in patients with SIRS / Sepsis

Graciela Libier Cabrera Rivera[1], Eduardo Antonio Ferat-Osorio[2], Constantino López-Macías[2], Armando Isibasi[2], Isabel Wong-Baeza[1], Lourdes Andrea Arriaga-Pizano[2]

[1]*Instituto Politécnico Nacional, Mexico City, Mexico*
[2]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
libier_9@hotmail.com; eduardoferat@prodigy.net.mx; constantino@sminmunologia.mx; isibasi@prodigy.net.mx; isa_wb@yahoo.com.mx; landapi@hotmail.com

Keywords: Sepsis, SIRS (for Systemic Inflammatory Response Syndrome), IL-6 (Interleukin 6), mIL-6R (membrane receptor), sIL-6 (soluble receptor)

Introduction: During acute systemic inflammation, such as SIRS (for Systemic Inflammatory Response Syndrome) or sepsis, cytokines and acute phase proteins are increased. IL-6 is one of such inflammatory soluble mediators which could be produced by monocytes, macrophages, dendritic cells (DCs), endothelial cells and hepatocytes. Because it induces IL-1β and TNF-α production and promotes neutrophils and macrophages maturation. IL-6 is consider as a pro-inflammatory mediator; however, in knockout mice to IL-6 (IL-6 -/-) with endotoxemia, an increased levels of the pro-inflammatory cytokines TNF-α, IL-1β, MCP-1, IFN-γ was observed, compared to wild type mice. Also, neutrophils infiltration to lung was exacerbated in IL-6 KO mice.

Signaling by receptor IL-6 soluble receptor (sIL-6R) activates MYD88 and NFKB promoting pro-inflammatory cytokine transcription, while mIL-6R (membrane receptor) activation, leads to JAK-STAT3 and IL-1RA. This type of signaling by mIL-6 has been related to a decrease in the induction of respiratory burst and degranulation by neutrophils, which is proposed to restrict the damage caused by excessive recruitment in an inflammatory process. So the dual role of IL-6 in inflammation could be partially explained by which IL-6R is engaged. Even in SIRS/sepsis IL-6 augmentation has been used as biomarker, no solid evidence exist about the dual response to IL-6 by leucocytes from these patients. Our aim was to determine sIL-6R in sera and mIL-6R on circulating phagocytes from patients with SIRS or sepsis.

Methodology: Peripheral blood samples (10 cc) were obtained from patients diagnosed with SIRS (n=2) or sepsis (n=10) and healthy volunteers (n=10). Immuno-staining

was carried out with monoclonal antibodies conjugated to fluorochrome, where IL-6R is included to characterize the expression of the membrane receptor in neutrophils (CD45low, CD14-, CD16+) and monocytes (CD45low, CD14+, CD16-). Samples were analyzed using a FACS AriaIIU flow cytometer. Soluble receptor (IL-6-sIL6R) in serum was quantified by ELISA technique.

Results: We observed no differences in sIL-6R levels among groups. This was also true for mIL-6R expression in both neutrophils (mIL-6R MFI in SIRS/sepsis vs in healthy, p= 0.441) and monocytes (mIL-6R MFI in SIRS/sepsis vs in healthy and mIL-6R MFI in SIRS/sepsis vs in healthy, p=0.006**). However, a significantly lower number of monocytes express mIL-6R in septic patients, but not in those with SIRS.

Conclusion: In sepsis, even each phagocyte cell may elicit the same response that the ones from healthy individuals, the pro-inflammatory role of IL-6 could prevail because the diminished numbers of cells expressing anti-inflammatory mIL-6R.

Antiinflamatory effect of a novel peptide ameliorate imiquimod-induced psoriasis-like skin in mice

Guaalupe Gutiérrez-González, Mario E Cancino-Díaz, Ivan Diaz-Toalá

Instituto Politécnico Nacional, Mexico City, Mexico
jeulis_23@yahoo.com.mx; mcancinodiaz@gmail.com; lugutierrez998@gmail.com

Keywords: Peptides, Psoriasis, immunology, phage display, chronic inflamation

Psoriasis is chronic inflammatory condition of the skin, and is characterized by thick erythematous plaques (also knows as plaque psoriasis or psoriasis vulgaris), silver scales, localized on extensor surfaces. Psoriasis affecting from 2% to 3% people worldwide and is rare in african people. Psoriasis is associated comorbid diseases such as cardiometabolic disorder, stroke, psoriatic arthritis, chronic kidney diseases. Psoriasis pathogenesis mechanism are described for the role of several cytokines and is considered a T-cell immune diseases, modified by genetic susceptibility meanly by genes that are involved in antigen presentation. The objetive of this work is the development of novel biofarmaceutical products that ameliorates chronic inflamation as psoriasis. Phage display technology involves producing libraries of 1010 peptides displayed on phage and this peptides can contain fixed residues that impose a structure. Beside peptides is an excellent alternative for drug development.

Using this phage display technology, we compared the cytokine receptor that models part of the pathogenesis in psoriasis with the phage display library and obtained eighteen bacterial clones. These eighteen bacterial clones were isolated and amplified to perform ELISA for specificity tests on the receptor above mencioned. The clones were sequenced and through BLAST it was possible to establish that the eighteen clones belonged to six different amino acid sequences, which have a motive sequence that is maintained between different species. The six amino acid sequences obtained were modeled by homology (Modeller) and then subjected to a molecular dynamics (VMD) to calculate the RMSD and deermine those sequences that achieve equilibrium, of which two sequences reached equilibrium and were the candidate sequences to submit them to docking. In the docking assay, the position in which the peptide binds to the receptor was determined. The clones were sequenced and through BLAST it was possible to establish that the eighteen clones belonged to six different amino acid sequences, which have a motive sequence that is maintained between different species. The six amino acid sequences obtained were modeled by homology (Modeller) and

then subjected to a molecular dynamics (VMD) to calculate the RMSD and deermine those sequences that achieve equilibrium, of which two sequences reached equilibrium and were the candidate sequences to submit them to docking. In the docking assay, the position in which the peptide binds to the receptor was determined.

Macrophage migration inhibitory factor (MIF) levels in gingival crevicular fluid, saliva and serum from subjects with periodontitis

Yveth Marlene Ortiz García[1], Trinidad García Iglesias[2], Gabriela Morales Velazquez[1], José Justino Cervantes Cabrera[1], Celia Guerrero Velazquez[1], Annie Riera Leal[2], Ana Lourdes Zamora Perez[1]

[1]University of Guadalajara, Guadalajara, Mexico
[2]Universidad de Guadalajara, Guadalajara, Mexico
ortizgamarlene@hotmail.com; trini.iglesias@gmail.com; gmove16@hotmail.com; just_7@hotmail.com; celiagv2001@yahoo.com.mx; annierieraleal78@yahoo.es; anazamora@gmail.com

Keywords: Gingival Crevicular Fluid, Inflammation, Periodontitis, Saliva, macrophage migration inhibitory factor (MIF)

Introduction: Periodontitis (PE) is an infectious that affects the teeth supporting as the result of the imbalance between the immunological interaction of the host and the flora of the dental bacterial plaque that colonizes the gingival sulcus (1).

The presence of biofilm and dental plaque initiates a destructive inflammatory process that leads to the formation of periodontal pockets (2), and this represents a well-characterized microbial change during the transition from periodontal health to periodontal disease (3).

Among these, the "red complex", consisting of Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola, is considered the most pathogenic microbial complex (4).

Cytokines are an important component in the inflammatory response in periodontitis and many studies have demonstrated that various proinflammatory cytokines are involved in the immunopathology of this disease (5).

The macrophage migration inhibiting factor (MIF) is an important effector cytokine of the innate immune system (6). It is expressed constitutively in a variety of immune and non-immune cells, promote leukocyte recruitment in sites of infection and inflammation. It is released rapidly in response to microbial products, proliferative signals and hypoxia (7, 8).

Due to functional characteristics, MIF may be involved in the immunopathology of periodontal disease. Previous studies have indicated the presence of this cytokine in

epithelial tissue of patients with PE (9), in gingival crevicular fluid (GCF) of subjects with induced gingivitis (10) and generalized aggressive periodontitis (11).

Objective: Determine MIF levels in gingival crevicular fluid, saliva and serum from subjects with PE.

Materials and Methods: This is cross-sectional study that was carried in the Health Sciences University Center of the University of Guadalajara.

The GCF, saliva and serum were obtained from subjects with PE (n= 30) and without PE (n=30).

All participants signed an informed consent before to their inclusion in the study and the periodontal status was evaluated by measuring the plaque index (PI), gingival bleeding index (BI), probing depth (PD) and clinical attachment level (CAL).

The concentrations of MIF were quantified by the enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (BioLegend LEGEND MAX™).

The results are expressed as means ±SD and means ±SE. Differences in MIF concentrations were evaluated using Mann–Whitney's U test for intergroup comparison and Wilcoxon test for intragroup comparison. A Pearson correlation was performed to test the relationship between MIF concentration and clinical status. All tests were performed using the Statistical Program for Social Sciences (SPSS v11.0) for Windows (SPSS, Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results: The age average of the group with and without PE was 43.6 ± 9.1 and 39.2 ± 7.2 years respectively.

In terms of clinical data (table 1), the group with PE presents significantly higher values than the group without PE (PI, BI, PD and CAL).

The concentrations of MIF in the group with PE in GCF, saliva and serum were 17.85±1.04, 72.94±10.90 and 9.91±1.65 ng/mL respectively. While in the group without PE were from 8.85±1.28, 31.5±5.76 and 7.08±0.58 ng/mL respectively.

The concentrations of MIF obtained in the three different fluids were compared between groups. Significantly increased levels of MIF in GCF (p=0.001) and saliva (p=0.001)

Table 1: General profile and clinical measurements of subjects from the study

Variables	Groups		
	1: Without PE	2: With PE	p value
(n)	30	30	
Age (years)	39.2±7.2	42.6±9.1	*ns*
Gender			
– Female (%)	15 (50)	15 (50)	
– Male (%)	15(50)	15(50)	
CAL (mm)	0.92±0.27	4.36±1.14	***0.001***
PD (mm)	1.18±0.43	3.44±0.89	***0.000***
BOP(%)	6.13±3.25	14.07±5.03	***0.000***
SP (%)	12.37±5.62	33.43±15.86	***0.000***

Gingival bleeding index (BI); Clinical attachment level (CAL); Sample size (n); Plaque index (PI); Probing depth (PD). Gender, SP and BOP data are expressed as percentages. Age, PD, CAL, BOP and SP values are expressed as mean SD. U de Mann Whitney test was used to compare clinical measurements.

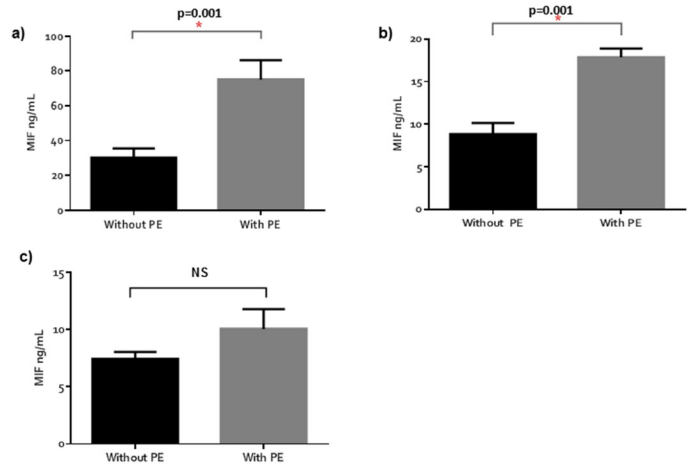


FIGURE 1: MIF concentrations in the group without PE and with PE. MIF comparison in: **a)** GCF; **b)** saliva **c)** serum. *Significant difference. NS: not significant difference. GCF: Gingival crevicular fluid; MIF: Inhabitor Factor of the Migration of Macrophages. PE: periodontitis.

were found in subjects with PE. Even that serum concentrations of MIF were higher in the group with PE compared with the group without PE, not significant differences were observed (p=0.509) (figure 1).

Intragroup comparisons of MIF concentrations were made between the different fluids of each study group and we found that MIF concentrations were significantly higher in GFC and saliva compared with serum in both study groups ($p < 0.05$).

A positive significant correlation ($p < 0.05$) was also observed between clinical parameters (SP, BOP, DP and CAL) and MIF concentrations in GFC (table 2).

Conclusion: Our results indicate that individuals with PE have higher concentrations of MIF in GCF and saliva; however in serum we did not observe significant difference. This could be due to the contact that both fluids have with the place where the development of the disease occurs and this could be corroborate with the positive correlation found with the clinical parameters and MIF concentrations in GCF.

Table 2: Correlation between clinical parameters and MIF concentrations in the different fluids

Variable	GCF MIF ng/mle		Salival MIF ng/ml		Serum MIF ng/ml	
	r	*p*	r	*p*	r	*p*
CAL	**0.60**	**0.000**	**0.29**	**0.034**	0.09	0.519
PD	**0.56**	**0.000**	0.25	0.071	0.16	0.249
Bl	**0.50**	**0.000**	0.20	0.133	0.03	0.786
PI	**0.40**	**0.004**	0.03	0.870	0.12	0.381

Gingival bleeding index (BI); GCF: gingival crevicular fluid; Clinical attachment level (CAL); Plaque index (PI); Inhibitor Factor of the Migration of Macrophages (MIF); Probing depth (PD). Spearman correlation; r: correlation coefficient. $p<0.05$ was considered statistically significant.

REFERENCES

1. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. Nat Rev Dis Primers. 2017; 3:17038.
2. Zijnge V, Ammann T, Thurnheer T, Gmür R. Subgingival biofilm structure. Front Oral Biol. 2012; 15: 1-16.
3. Curtis MA, Zenobia C, Darveau RP. The relationship of the oral microbiotia to periodontal health and disease. Cell Host Microbe. 2011; 10(4): 302-6.
4. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015; 15(1): 30-44.
5. Yucel-Lindberg T, Båge T. Inflammatory mediators in the pathogenesis of periodontitis. Expert Rev Mol Med. 2013; 5;15:e7.
6. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol. 2003; 3(10):791-800.
7. Guimaraes CC, Galvao JM, Araújo TA, Oliveira RN, Ferreira DC, Sousa V, Veríssimo J. Macrophage Migration Inhibitory Factor (MIF): Biological Activities and Relation with Cancer. Pathol Oncol Res. 2017; 2:235-244.

8. Grieb G, Merk M, Bernhagen J, Bucala R.Macrophage migration inhibitory factor (MIF): a promising biomarker. Drug News Perspect. 2010; 23(4):257-64.
9. Li X, Lan HY, Huang XR, Zhang C, Jin LJ. Expression profile of macrophage migration-inhibitory factor in human gingiva and reconstituted human gingival epithelia stimulated by Porphyromonas gingivalis lipopolysaccharide. J Periodontal Res. 2013; 48(4):527-32.
10. Nonnenmacher C, Helms K, Bacher M, Nüsing RM, Susin C, Mutters R, Flores-de-Jacoby L, Mengel R. J Dent Res. 2009. 88(7):639-643.
11. Lira-Junior R, Öztürk VÖ, Emingil G, Bostanci N, Boström EA. Salivary and Serum Markers Related to Innate Immunity in Generalized Aggressive Periodontitis. J Periodontol. 2017; 28:1-13.

High expression of TLR7 and TLR9 in Rheumatoid Arthritis

Elsy Janeth Ramos[1], Yadira Bastian[1], Martin Zapata[2], Jose Antonio Enciso-Moreno[1]

[1]*Unidad de Investigación Biomédica de Zacatecas (IMSS), Zacatecas, Mexico*
[2]*Autonomous University of Zacatecas, Zacatecas, Mexico*
elsy.jrg@gmail.com; ybastianhe@conacyt.mx; martinzz1@yahoo.com; enciso_2000@yahoo.com

Keywords: Autoimmunity, Rheumatoid arthritis, innate immunity, TLRs (Toll-like receptors), immflamatory desease

Introduction: Rheumatoid arthritis (RA) is defined as a chronic systemic disease that cause damage to the joints, provoking deformity and disability to humans. In Mexico, it is estimated that RA has a prevalence of 1.6% with an annual medical cost of approximately US $ 2334.00 per patient.

During the pathological process of RA occurs synovitis, synovial hyperplasia with neovascularization and joint inflammation that leads to immobility and joint pain. The inflammation provoked in RA is the main symptom responsible for the pain and the sensation of stiffness that patients present in the morning.

It has been described that the expression of Toll-like receptors (TLR's) is elevated in synovial tissue of patients with RA. It has been suggested the altered expression of TLRs in synovial tissue indicates a response to endogenous alarm molecules and molecular patterns associated with pathogens (PAMP's), which would contribute to the perpetuation of inflammation characteristic of the disease. Multiple cytokines, such as TNF-α, IL-1β, IL-6 may also contribute to the inflammatory processes during the pathogenesis of RA.

To date, changes in the expression of TLRs in the early phase of RA (eRA) and towards the progression to established RA (cRA) have not been studied. From this point of view, the early and accurate detection of the altered levels of molecules such as the TLRs, could have an enormous utility as biomarkers in the early diagnosis of this disease in high risk subjects. An early and timely diagnosis based on biomarkers is necessary to give a proper treatment to ART patients, when treatment is more beneficial.

The main goal is to evaluate the expression levels of TLR7 and TLR9 in RA patients.

Material and Methods: In a cross-sectional study with humans and according to the 2010 ACR/EULAR classification criteria, four groups of individuals were analyzed: healthy subjects (HC), ACCP+ asymptomatic subjects (ACCP+), early RA patients (eRA) and established RA patients (cRA). Whole blood samples were obtained and total RNA was extracted to perform qPCR assays for the TLR7 and TLR9 genes. Serum was used for the determinations of citrullinated anti-peptide antibodies (ACCP).

Results: TLR7 expression was found elevated in cRA and eRA patients and in ACPP+ respect to HC subjects (Figure 1). TLR9 gene expression was elevated only in cRA and ACCP+ with respect to HC (Figure 2).

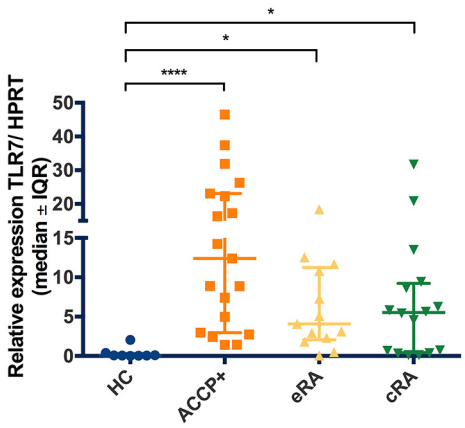


FIGURE 1: Relative expression of the TLR7 gene with respect to the constitutive HPRT gene in the groups of healthy controls, ACCP + asymptomatic subjects, early RA patients and established RA patients. HC: healthy controls, ACCP+: ACCP + asymptomatic subjects, eRA: early Rheumatoid Arthritis, cRA: established Rheumatoid Arthritis.

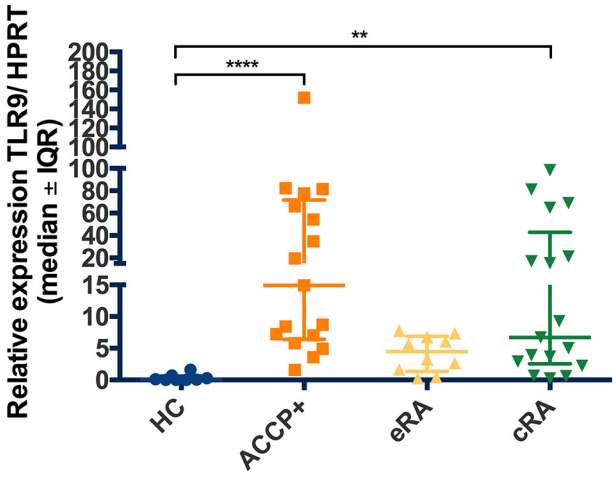


FIGURE 2: Relative expression of the TLR9 gene with respect to the constitutive HPRT gene in the groups of healthy controls, ACCP + asymptomatic subjects, early RA patients and established RA patients. HC: healthy controls, ACCP+: ACCP + asymptomatic subjects, eRA: early Rheumatoid Arthritis, cRA: established Rheumatoid Arthritis.

Conclusions: TLR7 and TLR9 gene expression is elevated in patients with RA (early and established) and in ACCP+. TLRs may be considering a good biomarker for RA given the increase in expression occurs since ACCP+ high risk group but not in HC subjects.

Anti-TNF treatment in patients with inflammatory bowel disease impairs pro-inflammatory cytokines secretion by circulating leucocytes

Alma Verónica Moreno González, Eduardo Ferat Osorio, Uriel Guadarrama Aranda, Ilka Berenice Boscó Garaté, Rosalba Moreno Alcantár, Arturo Mayoral Zavala, Constantino III Roberto López Macías, Armando Isibasi Araujo, Lourdes Andrea Arriaga Pizano

Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
almav_mg@hotmail.com; eduardoferat@prodigy.net.mx; aranda@comunidad.unam.mx; ibosco45@hotmail.com; rosalba_moreno@yahoo.com.mx; gastroamz@yahoo.com.mx; constantino@sminmunologia.mx; isibasi@prodigy.net.mx; landapi@hotmail.com

Keywords: Inflammation, Inflammatory Bowel Diseases, Leukocytes, proinflammatory cytokines, anti-TNF-α

Introduction: The inflammatory response involves the activation of the pattern recognition receptors (PRRs) that will induce the production and secretion of cytokines and chemokines that activate endothelium and leukocytes. Under normal conditions the inflammatory response is limited. Sometimes, these pro-inflammatory mediators abnormally prevail and give rise to chronic inflammatory processes, as in Inflammatory Bowel Disease (IBD). IBD is the term that includes two pathological entities: ulcerative colitis (UC) and Crohn's disease (CROHN). IBD is characterized by recurrent chronic inflammation, alternating periods of remission with exacerbations, which are related to the overproduction of anti-inflammatory cytokines such as TNF (tumor necrosis factor). The biological therapy includes anti-TNF antibodies known as infliximab and adalimumab. These antibodies bind to both soluble (TNF) and membrane TNF (mTNF), preventing their binding to the TNF receptors: TNFR1 and TNFR2. In intestine, infliximab and adalimumab decrease the inflammatory infiltrate, extracellular matrix degradation, enhance regulatory macrophages (which limits T cells proliferation), and significantly reduce the expression of TNFRs. Anti-TNF therapy induces and maintains the remission of symptoms in 50 to 80% of the cases. It is indicate in patients non-responsive to conventional treatment. Even a lot of information is available about the mechanism explaining the therapeutic effects of anti-TNF therapy, little is none about the effect it has in normal TNF functions. It is suggested that beyond therapeutic effect, an immunosupression state is generated, since among the non-desirable effects of this therapy are: diminished responses to vaccination reactivation of tuberculosis and increased susceptibility to systemic infections and their complications, and to lymphoproliferative diseases development. Our aim was to determine the pro-inflammatroy

cytokine production in leucocytes from patients with IBD in treatment with anti-TNF antibodies.

Methodology: Peripheral blood (10 cc), drawned in heparin tubes, was obtained from patients with IBD in treatment with and without anti-TNF antibodies. Serum was separated for cytokine quantification by immunoassay. Leucocytes were isolated and stimulated or not with LPS [100ng/mL] for 4 hours with Brefeldin-A (BFA-A)[10µg / mL] (for intracellular cytokine detection) or without BFA-A (for secreted cytokine secretion). By flow cytometry, monocytes were identified as CD45med(anti-hCD45/PO) and CD14+ (anti-hCD14/PECy7) and anti-IL-1β/PB, anti-IL-8/APC, anti-IL-6/FITC and anti-TNF/PE were used for intracellular cytokine detection after cell membrane permeabilization. Selection of positive populations was based on fluoresecence minus one control (FMO). All samples were analyzed using a FACSAria IIU flow cytometer(BDB). Data were analyzed with Infinicyt sorfware v.1.8 (Cytognos). In both serum and supernantants from stimulated cells the cytokines TNF, IL-1b, IL-6, IL-8, IL-12 and IL-10 were quantified by immunoassay.

Results: Thirty-five patients with IBD were included in this study. Sixteen have CROHN (10 with anti-TNF and 6 with conventional therapy), nineteen have UC (7 with anti-TNF and 12 with conventional therapy). As expected, TNF was significantly diminished in serum from patients with anti-TNF treatment ($p<0.03$).

Non-significant differences were found for cytokine production when determined intracelullarly. However, when quantifying secreted cytokines (TNF, IL-1b, IL-6) in response to LPS (100ng / mL 6 and 24 hrs post-stimulus), it is significantly ($*p<0.05$) lower than that detected in leukocyte supernatant from patients with anti-TNF compared to those receiving conventional therapy.

Conclusion: In patients with IBD, anti-TNF treatment does not compromises pro-inflammatory cytokines production but impairs cytokine secretion.

Mechanisms underlying the immunomodulatory properties of GK-1

Jacquelynne Cervantes-Torres[1], Laura Montero[1], Lisandro Sánchez[1], Diana Torres[1], Rene Segura-Velazquez[2], Edda Sciutto[1], Gladis Fragoso[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
otra_jbct@yahoo.com.mx; lau_mlcrl@yahoo.com; lisandro@unam.mx; dianatg@comunidad.unam.mx; realselab@gmail.com; edda@unam.mx; gladis@unam.mx

Keywords: Antigen presenting cell, TLR signaling, nfkb pathway, MAPK pathway, GK-1 peptide

GK-1 is an 18-amino-acid peptide that improves influenza vaccine's immunogenicity through the activation of dendritic cells (DC), favoring the synthesis of proinflammatory cytokines and chemokines and resulting in an efficient presentation of antigens. GK-1 also exhibits anti-tumoral properties. We have shown that intravenous administration of GK-1 increased the survival in 4T1 mammary tumor-bearing mice, decreasing tumor growth and the metastatic burden.

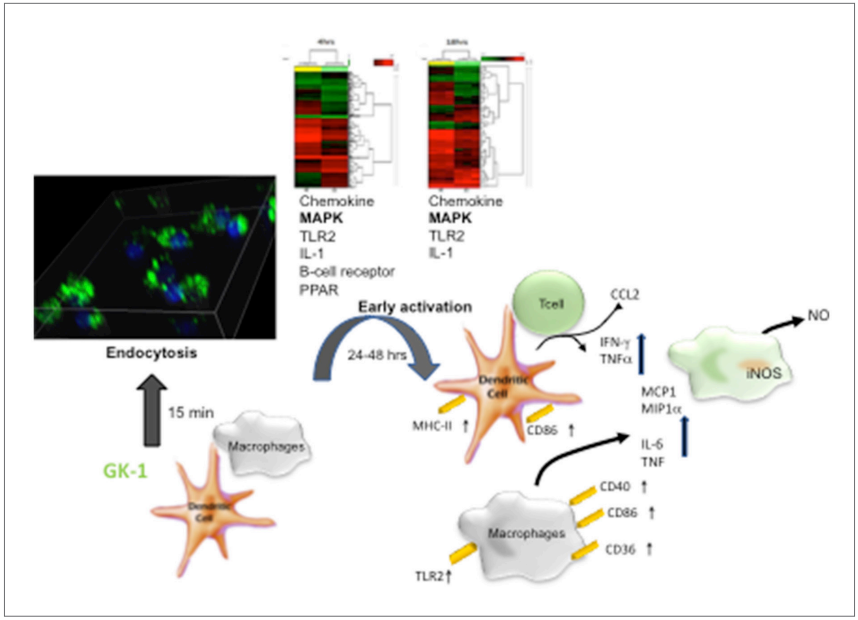
In this study the capacity of GK-1 to activate bone marrow derived dendritic cells (BMDC) as well as peritoneal macrophages was demonstrated. Thus, the cellular uptake of the peptide, its effect on the phagocytic properties and the intracellular events in antigen presenting cells were evaluated in order to identify possible mechanisms that may underlie its immunomodulatory properties.

Peritoneal macrophages treated with GK-1 increased their phagocytic activity. In a microarray analysis, it was found that GK-1 induced an early pro-inflammatory gene expression profile which is downregulated 18 hrs after the stimuli. Analysis of the up-regulated genes revealed that pro-inflammatory cytokines, chemokines, MAPK and TLR signaling pathways were those most importantly affected. Furthermore, the participation of the MAP kinases was confirmed through the evaluation of the phosphorylation of p38 and ERK1/2.

Confocal microscopy and flow cytometry analysis support the involvement of endocytosis as the major route for the internalization of GK-1 on BMDC in a kinetic that is saturated 45 minutes after treatment. Activation of these BMDC seems to be mediated

by the interaction with a TLR, favoring the early translocation to the nucleus of NF-kb (p65) through a Myd88 and TLR2-dependent pathway.

In conclusion, these findings elucidate the probably mechanisms by which GK-1 acts as immunostimulatory peptide.



Nlrp1b1 inflammasome negatively modulates obesity-induced inflammation

Jonathan Salazar-León[1], Ana Laura Valdez-Hernández[1], Sara García-Jiménez[2], Leonor Pérez-Martínez[1], Gustavo Pedraza-Alva[1]

[1]Instituto de Biotecnologia, Cuernavaca, Mexico
[2]Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
salazarleon29@gmail.com; analaura@ibt.unam.mx; saragarcia@uaem.mx; leonor@ibt.unam.mx; gustavo@ibt.unam.mx

Keywords: Inflammation, Inflammasome, caspase-1, IL-1b, IL-18, NLRP

Obesity is a global public health problem, resulting from a chronic imbalance between consumption and energy expenditure. Obesity promotes the development of alterations comprising the metabolic syndrome and type 2 diabetes (1). Recently, it has been shown that chronic inflammation in the adipose tissue is critical for the pathogenesis associated to obesity [2,3,4]. This inflammatory process is characterized by the presence of activated macrophages in the adipose tissue that produce and secretes inflammatory cytokines such as IL-1β and IL-18 (5,6,7,8). The maturation and secretion of these cytokines in response to the lipid excess, involves the assembly and activation of a cytosolic multiprotein complex called inflammasome, constituted by the NLRP3 receptor, and the adaptor molecules such as ASC and Caspase-1 (9,10,11). Using animals deficient for any of these molecules, make clear that the inflammatory response triggered by the NLRP3 inflammasome is associated to obesity, since the result of this deficiency as is insulin resistance and therefore type II diabetes decrease (12,13,14).

However, given that i) the high fat diet (HFD)-induced caspase-1 activation in the adipose tissue of the NLRP3 knockout animals is not completely abrogated (12); ii) the NLRP3 deficient mice are in the C57BL/6 background, a strain susceptible to develop alterations in the glucose metabolism when fed with a HFD (15,16); iii) unlike C57BL/6 mice, Balb/c mice are resistant to gain weight and develop glucose metabolism alterations in response to a HFD (17), even though iv) the Nlrp3 genes between these two mouse strains is 100% similar. Given this background we speculated that other inflammasomes may contribute to the pathophysiology of obesity (18).

In this work, we show that the NLRP1B1 inflammasome protects C57BL/6 mice from developing glucose intolerance and insulin resistance when fed with a HFD, by reducing the infiltration of immune cells to adipose tissue, as well as the levels of active Caspase-1, and the levels of circulating IL-1b. Additionally, the NLRP1B inflammasome also protected against dyslipidemia and hepatic steatosis in obese mice. Together, our

results indicate that the NLRP1B1 inflammasome has a protective function against developing alterations in glucose and lipid metabolism in an obese mice model.

REFERENCES

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus--present and future perspectives. Nat Rev Endocrinol. 2012;8(4):228-236. doi:10.1038/nrendo.2011.183.
2. Anderson EK, Gutierrez DA, Hasty AH. Adipose tissue recruitment of leukocytes. Curr Opin Lipidol. 2010;21(3):172-177. doi:10.1097/MOL.0b013e3283393867.
3. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(December):860-867. doi:10.1038/nature05485.
4. Cinti S, Mitchell G, Barbatelli G, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res. 2005;46(11):2347-2355. doi:10.1194/jlr.M500294-JLR200.
5. Maedler K, Sergeev P, Ris F, et al. Glucose-induced beta cell production of IL-1 beta contributes to glucotoxicity in human pancreatic islets. J Clin Invest. 2002;110(6):851-860. doi:10.1172/JCI200215318.Introduction.
6. Böni-Schnetzler M, Donath MY. Increased IL-1β activation, the culprit not only for defective insulin secretion but also for insulin resistance? Cell Res. 2011;21(7):995-997. doi:10.1038/cr.2011.85.
7. Hung J, McQuillan BM, Chapman CML, Thompson PL, Beilby JP. Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. Arterioscler Thromb Vasc Biol. 2005;25(6):1268-1273. doi:10.1161/01.ATV.0000163843.70369.12.
8. Zorrilla EP, Sanchez-Alavez M, Sugama S, et al. Interleukin-18 controls energy homeostasis by suppressing appetite and feed efficiency. Proc Natl Acad Sci U S A. 2007;104(26):11097-11102. doi:10.1073/pnas.0611523104.
9. Schroder K, Tschopp J. The Inflammasomes. Cell. 2010;140(6):821-832. doi:10.1016/j.cell.2010.01.040.
10. Lamkanfi M, Kanneganti T-D. The inflammasome: A remote control for metabolic syndrome. Cell Res. 2012;22(7):1095-1098. doi:10.1038/cr.2012.55.
11. Wen H, Gris D, Lei Y, et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat Immunol. 2011;12(5):408-415. doi:10.1038/ni.2022.
12. Vandanmagsar B, Youm Y-H, Ravussin A, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med. 2011;17(2):179-188. doi:10.1038/nm.2279.
13. Stienstra R, Joosten LAB, Koenen T, et al. The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. Cell Metab. 2010;12(6):593-605. doi:10.1016/j.cmet.2010.11.011.
14. Stienstra R, van Diepen JA, Tack CJ, et al. Inflammasome is a central player in the induction of obesity and insulin resistance. Proc Natl Acad Sci. 2011;108(37):15324-15329. doi:10.1073/pnas.1100255108.
15. Surwit RS, Feinglos MN, Rodin J, et al. Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and mice. Metabolism. 1995;44(5):645-651. doi:10.1016/0026- 0495(95)90123-X.
16. Surwit RSS, Kuhn CMM, Cochrane C, McCubbin J a., Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. Diabetes. 1988;37(9):1163-1167. doi:10.2337/diabetes.37.9.1163.
17. Montgomery MK, Hallahan NL, Brown SH, et al. Mouse strain-dependent variation in obesity and glucose homeostasis in response to high-fat feeding. Diabetologia. 2013;56(5):1129-1139. doi:10.1007/s00125-013-2846-8.
18. Murphy AJ, Kraakman MJ, Kammoun HL, et al. IL-18 Production from the NLRP1 Inflammasome Prevents Obesity and Metabolic Syndrome. Cell Metab. 2016;23(1):155-164. doi:10.1016/j.cmet.2015.09.024.

Role of estrogen and progesterone in gene expression of inflammasome regulator molecules

Oscar Humberto López-Portales[1], José Martin Murrieta Coxca[1], José Martin Murrieta Coxca[2], Emmanuel Vargas Pérez[1], Juan Carlos Cancino-Diaz[1], Sandra Rodríguez-Martínez[1]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[2]Friedrich Schiller Universität Jena, Jena, Germany

ohlp_7@hotmail.com; coxca_qfb@hotmail.com; coxca_qfb@hotmail.com; enemac_25@hotmail.com; jccancinodiaz@hotmail.com; sandrarodm@yahoo.com.mx

Keywords: Uterus, regulation, Inflammasome, mouse model, estrogen and progesterone

Inflammation comprises a non-specific immune response triggered by a harmful stimulus, i.e. pathogen infection, cell damage, mechanical stress etc. This process is related to fertility and reproduction, regarding reproductive cycle (ovulation and menses) but also early stages of pregnancy (decidualization and embryo implantation) and labor stage [1,2]. In health, implantation and labor stages are predicted to be a tightly regulated inflammatory event due to an increase in leukocyte infiltrate and the production of pro-inflammatory molecules in the uterus [3].

Interleukin-6 (IL-6), TNF-α, and IL-1 family cytokines like IL-1β and IL-18 have been described as the main participants in inflammation during the events cited above. Inflammasomes play a transcendental role on the onset of the inflammatory environment since these cytosolic, multi-unit signaling complexes are in charge of the maturation and release of IL-1β and IL-18 to the extracellular milieu through pyroptosis [4,5,6].

One of the most studied complexes is the NLRP3 inflammasome, which consists of the pyrin domain-containing NLR protein (NLRP3), the adaptor molecule apoptosis-associated speck-like protein containing CARD (ASC) and the proinflammatory caspase-1. NLRP3 receptor can be activated by a wide array of stimulus including pathogen- and endogenous-derived activators as well as sterile exogenous activators [7]. Inflammasome triggering provides a means of defense against pathogens, but its dysregulation is linked to the development of autoimmune and autoinflammatory diseases, and in terms of reproduction it is associated to endometriosis, polycystic ovary, intrauterine growth restriction, preterm birth and miscarriage, amongst other issues [2]. Therefore, the tight control of inflammasome assembly and signaling is crucial to

allow the immune system to initiate antimicrobial and inflammatory responses while avoiding overt tissue damage.

Regulation of NLRP3 inflammasome activation spans from transcription induction to post-translational modifications [8]. Additionally, interaction with other proteins sets up another level of regulation. Through a direct mechanism, the kinase NEK7 and the transcriptional factor NRF2 interact with NLRP3 and ASC respectively, to promote inflammasome assembly. At the same time, NRF2 indirectly antagonizes inflammasome activation via induction of antioxidant genes expression. On the other hand, the thioredoxin-interacting protein, TXNIP, enhances inflammasome activation both through an indirect mechanism, and through direct physical interaction [9,10,11].

In our laboratory, we used a model of ovariectomized (OVX) mice to analyze the expression of proinflammatory cytokines in the absence of sexual hormones, mainly estrogen and progesterone in whole uterine tissue. We observed differences in IL-1β and IL-18 transcripts between OVX mice picked up at diestrous or estrous phases of estral cycle for surgical intervention (data not shown) which led us to hypothesize that some other endogenous factors still control inflammasome activity besides estrogen and progesterone; namely the regulatory molecules NEK7, NRF2 and TXNIP.

We assessed the expression of pro–IL-1β together with the previously mentioned regulatory proteins ex vivo in the uterus of BALB/c mice under basal conditions and with estrogen or progesterone administration. For this purpose, we dissected the uteri of sexually mature mice during the diestrous phase of estral cycle. After carefully removing connective and fat tissue and the vaginal tract, whole uterine tissues were placed individually in 6-well plates and incubated in DMEM with 10% FBS at 37°C for 12 or 24 hours. Each group containing samples from 3 mice were divided as following: i) Non-stimulated, ii) Stimulated with 3 different concentrations of estrogen (0.18μM, 1.8μM and 18μM), iii) Stimulated with 3 different concentrations of progesterone (160μM, 320μM and 640μM). After the stimulation period, uterine tissues were collected, and liquid nitrogen frozen for pulverization in TRIzol reagent for RNA isolation. Genetic material was used in RT-PCR assays to evaluate the expression of NEK7, NRF2, TXNIP and pro–IL-1β. We used β-actin as housekeeping control gene.

As a result, we observed that estrogen addition greatly increased transcription of NEK7, NRF2 and TXNIP within 12 hours of stimulation and that this coincided with higher levels of pro–IL-1β transcript. Conversely, at 24 hours of stimulation with estrogen, transcription of NRF2 was reduced and NEK7 and TXNIP remained unaltered with an apparent agreement of pro–IL-1β transcript levels. In contrast, stimulation with progesterone at 12 hours showed no difference in transcription of NRF2, TXNIP and pro–IL-1β compared to basal conditions but did show a reduction in NEK7 transcript

levels. Lastly, within 24 hours of progesterone stimulation, NEK7 and NRF2 transcription decreased, TXNIP remained unaltered and pro–IL-1β increased considerably.

As a conclusion, it is suggested that estrogen works as an inflammatory stimulus that also promotes transcription of genes that upregulate inflammasome assembly, while progesterone has a similar effect at a late time point but in this case the regulatory proteins interestingly showed a reduction. We are aware that further studies need to be done, especially in the matter of protein production and also regarding to the behavior of the regulatory molecules in the rest of estral cycle phases and during pregnancy.

REFERENCES

[1] M. Nadeau-Vallée et al., "Sterile inflammation and pregnancy complications: a review," Reproduction, vol. 152, no. 6, pp. R277–R292, 2016.

[2] M. PrabhuDas et al., "Immune mechanisms at the maternal-fetal interface: perspectives and challenges," Nat. Immunol., vol. 16, no. 4, pp. 237–243, 2015.

[3] J. M. Murrieta-Coxca et al., "Estrous cycle and gestational age-dependent expression of members of the interleukin-36 subfamily in a semi-allogeneic model of infected and non-infected murine pregnancy," Front. Immunol., vol. 7, no. SEP, pp. 1–10, 2016.

[4] F. Martinon, K. Burns, and J. Tschopp, "The Inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-β," Mol. Cell, vol. 10, no. 2, pp. 417–426, 2002.

[5] P. Broz and V. M. Dixit, "Inflammasomes: mechanism of assembly, regulation and signalling.," Nat. Rev. Immunol., vol. 16, no. 7, pp. 407–20, 2016.

[6] S. Patel, "Inflammasomes, the cardinal pathology mediators are activated by pathogens, allergens and mutagens: A critical review with focus on NLRP3," Biomed. Pharmacother., vol. 92, pp. 819–825, 2017.

[7] J. Davis, BK., Wen, H., Ting, "The Inflammasome NLRs in Immunity, Inflammation, and Associated Diseases.," Annu Rev Immunol, pp. 707–735, 2011.

[8] B. Poudel and P. Gurung, "An update on cell intrinsic negative regulators of the NLRP3 inflammasome," J. Leukoc. Biol., no. September 2017, pp. 1–13, 2018.

[9] S. M. U. Ahmed, L. Luo, A. Namani, X. J. Wang, and X. Tang, "Nrf2 signaling pathway: Pivotal roles in inflammation," Biochim. Biophys. Acta - Mol. Basis Dis., vol. 1863, no. 2, pp. 585–597, 2017.

[10] Y. He, M. Zen, D. Yang, B. Motro, and G. Núñez, "NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux," Nature, vol. 530, no. 7590, pp. 354–357, 2016.

[11] R. Zhou, A. Tardivel, B. Thorens, I. Choi, and J. Tschopp, "Thioredoxin-interacting protein links oxidative stress to inflammasome activation," Nat. Immunol., vol. 11, no. 2, pp. 136–140, 2010.

Mycobacterium tuberculosis promotes IL-21 and IL-21R expression in alveolar macrophages under in vitro infection

Marco Polo Peralta, Brenda Brenda Marquina, Dulce Adriana Mata, Rogelio Hernandez Pando

Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
marcopolo_308115163@comunidad.unam.mx; brenda.marquina@gmail.com; dulmat@yahoo.com.mx; rhdezpando@hotmail.com

Keywords: Macrophages, in vitro, mouse model, IL-21, Pulmonary tuberculosis (PTB)

IL-21 is a cytokine that was characterized for the first time in 2000. It binds to its receptor (IL-21R), which needs to be coupled to gamma chain to activate several intracellular signals.[1] It's functions have been linked to proliferation, differentiation and redirection of immune responses in distinct entities, such as intracellular infectious diseases.[2] In the case of Pulmonary Tuberculosis (PTB), its presence and production has been related to a protective function, as a consequence of TH1 profile activation with a highly production of TNF-a and IFN-g from TCD-8 lymphocytes as in NK cells as well [3,4]. However, until now the production of this cytokine has only been documented in TFH (follicular) lymphocytes, TH17, and NKT cells.[5] In a model of progressive pulmonary tuberculosis in BALB /c mice infected with prototype strain H37Rv, we have observed an increase in the expression of IL-21 and its receptor by q-PCR and immunohistochemistry in lung tissue at days 21 and 28 days' post- infection and a reduction on days 60 and 120, which is similar to Th1 cytokine kinetics that is protective against Mycobacterium tuberculosis.[6] Furthermore, the same response was seen in vitro infecting murine alveolar macrophages (MHS) with prototype strain H37Rv or incubating them with Muramyl dipeptide (MDP). Thus, we show for the first-time the IL-21 expression in macrophages infected with mycobacteria suggesting that protection in this intracellular infection is mediated by multiple innate immunity mechanisms including IL-21 production.

REFERENCES

1) Asao, Hironobu et al "Cutting Edge: The Common γ-Chain Is an Indispensable Subunit of the IL-21 Receptor Complex." The Journal of Immunology 167.1 (2001): 1-5. Web. 31 Jan. 2018.

2) Jesse G. Toe, Marc Pellegrini, Tak Wah Mak; "Promoting immunity during chronic infection—The therapeutic potential of common gamma-chain cytokines". Molecular Immunology, Volume 56, Issues 1–2,2013.

3) Booty M.G., Barreira-Silva P., Carpenter S.M., Nunes-Alves C., Jacques M.K., Stowell B.L., Jayaraman P.;" IL-21 signaling is essential for optimal host resistance against Mycobacterium tuberculosis infection" Scientific Reports, 6 , art. no. 36720 (2016)

4) Venkatasubramanian, Sambasivan et al. "IL-21-Dependent Expansion of Memory-like NK Cells Enhances Protective Immune Responses against Mycobacterium Tuberculosis." Mucosal immunology 10.4 (2017): 1031–1042. PMC. Web. 31 Jan. 2018.

5) Sue M. Liu, Cecile King; IL-21–Producing Th Cells in Immunity and Autoimmunity. The Journal of Immunology October 1, 2013, 191 (7) 3501-3506.

6) Hernández-Pando R, Orozcoe H, Sampieri A, et al. Correlation between the kinetics of Th1, Th2 cells and pathology in a murine model of experimental pulmonary tuberculosis. Immunology. 1996;89(1):26-33.

Extracellular vesicles released by Mycobacterium tuberculosis infected J774A.1 macrophages induce a reduction of bacterial load in a murine progressive pulmonary tuberculosis model

Mariano García Martinez[1], Violeta Alvarez-Jimenez[1], Luis Vazquez Flores[1], Jessica Castañeda Casimiro[1], Miguel Ibañez-Hernandez[2], Jeanet Serafin López[1], Rogelio Hernandez-Pando[3], Dulce Adriana Mata- Espinosa[3], Jorge Alberto Barrios-Payan[3], Luvia Enid Sanchez-Torres[1], Sergio Estrada-Parra[1], Rommel Chacon-Salinas[1], Iris Estrada-Garcia[1]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[2]Departamento de Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[3]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

mekryn@hotmail.com; vio_ajd@yahoo.com.mx; valu_868@hotmail.com; jcastanc@gmail.com; mibanez_01@hotmail.com; jeaserafin@hotmail.com; rhdezpando@hotmail.com; dulmat@yahoo.com.mx; qcjbp77@yahoo.com.mx; luviasanchez@hotmail.com; sestradap07@hotmail.com; rommelchacons@yahoo.com.mx; iestrada5@hotmail.com

Keywords: Mycobacterium tuberculosis, extracellular vesicles, Mycobacterial antigens, Colony forming units, J774A.1 murine macrophage, tuberculosis mouse model

Macrophages of the cell line J774A.1 release extracellular vesicles (EVs) spontaneously (Spont-EV). Infection of J774A.1 with Mycobacterium tuberculosis H37Rv (Mtb) induce the release of EVs with a modified protein and lipid content (Mtb-EVs). Basically Mtb-EVs have mycobacterial antigens and an increase in the amount of phosphatidylserine (PS) in comparison with Spont-EVs. In vitro, EVs reduced the phagocytosis of Mtb by J774A.1 cells, in particular Mtb-EVs showed the greatest reduction. In vivo, using a murine progressive pulmonary tuberculosis model (Balb/c infected with Mtb), EVs administered three times a week (2.5 micrograms (total protein content) intraperitonealy) at day 60 post-infection (p.i.), showed that bacterial loads - measured as colony forming units (CFU)- decreased in the lungs after 60 days of treatment (i.e. 120 days p.i.). The best effect was observed with Mtb-EVs, that induced an statistically significant reduction of UFC (92%) in comparison with the untreated infected mice. However, unexpectedly Spont-EVs also reduced the bacterial load (71%). Age of Mtb-EVs affected the results of bacterial load, since the greatest reduction was observed when Mtb-EVs were used within one week after purification (i.e. fresh). Probably

these results indicate that those mycobacterial antigens present in the fresh Mtb-EVs preparation may induce a protective immune response that translates into a better control of Mtb bacterial loads. Although these results are interesting, more work is needed to further confirm this hypothesis. Also further experiments combining Mtb-EVs with antituberculous drugs should be performed, in order to assess if they have a synergistic or an additive effect.

NLRP3 inflammasome activity is not regulated by NLRP10 expression in THP-1 macrophage-like cells infected by dengue virus

Ma Isabel Salazar, Inci Enid Ramírez-Bello, Gloria León-Ávila, Diana Alhelí Domínguez-Martínez

Instituto Politécnico Nacional, Mexico City, Mexico
maisabelsalazar@gmail.com; rabein28@hotmail.com; leonavila60@yahoo.com.mx; alheli43@hotmail.com

Keywords: Macrophages, Inflammasome, proinflammatory cytokines, Dengue infection, NLRP10

Human cells sense infections through a series of proteins known as Pattern Recognition Receptors (PRRs). Among these, there is a set of intracellular receptors known as NLRs (NOD-like receptors), from which NLRP3 is a key player in innate immunity and inflammatory processes. Recently, NLRP3 has been associated to the immune response of macrophages and platelets against dengue virus (DENV).

An important deregulation in numerous cytokines is observed in patients with severe dengue. However, the origin and the mechanisms underlying this phenomenon is not well understood. A question remains, how is that important effectors intervening in liberation of proinflammatory cytokines, such as NLRP3 are circumvented during the infection with DENV?

The inflammasome is responsible of triggering the liberation of two important proinflammatory cytokines: IL-1β and IL-18, making it important for us to understand how is this activity normally controlled and how is it altered during infections with DENV. In other disorders like Alzheimer´s disease, one of the molecules that seems to be crucial for the downregulation of NLRP3 is NLRP10. Taking this in account, we examined the expression of NLRP10 in basal conditions and in response to agonists and antagonists of the NLRP3 in DENV-infected THP-1 macrophage-like cells. The activation or inhibition of the NLRP3 inflammasome was indirectly determined by the quantification of IL-1β levels in the supernatants of treated cells (Figure 1).

The expression of NLRP10 was assessed in different experimental groups of THP-1 cells that were previously treated with LPS to stimulate the basal expression of NLRP10 (Figure 2). The experimental groups of cells were treated as follows: 1) Nigericin, an NLRP3 inflammasome agonist, 2) DENV infection and 3) IFN-β, an inflammasome antagonist, followed by DENV infection. Nigericin, produced a reduction in the expression of NLRP10. Meanwhile, DENV and IFN-β-DENV treatment produced

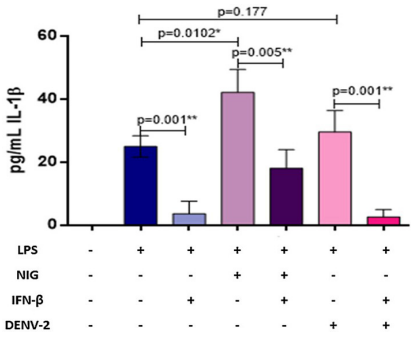


FIGURE 1.

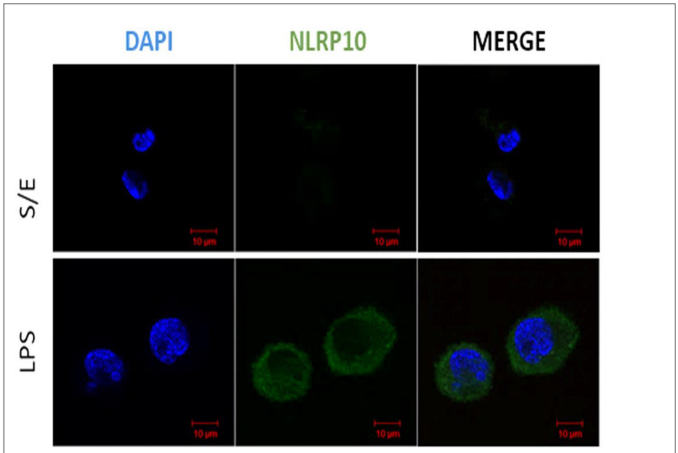


FIGURE 2.

an increase in NLRP10 expression and a relocalization of this protein to singular subcellular structures that were not characterized in this work. Contrary to what was expected, NLRP10 is not a negative regulator of the NLRP3 inflammasome in DENV-infected macrophages.

Strikingly, during confocal microscopy analyses of the localization of the viral NS1 protein, some co-localization with NLRP10 molecule was detected. However, any physical interaction between these two proteins must be verified in subsequent studies.

Soluble CD14 (sCD14) and Lipopolysaccharide Binding Protein (LBP) in celiac disease

Silvia Ferreira, Vivian Gimenez, María Mercedes Carpinelli, Patricia Langjahr

Universidad Nacional de Asunción, San Lorenzo, Paraguay, Paraguay
silviacfm@hotmail.com; viviangb@gmail.com; marichacarpinelli@hotmail.com; plangjahr@gmail.com

Keywords: Celiac Disease, Inflammation, innate immunity, LBP, soluble CD14

Introduction: Celiac Disease (CD) is an autoimmune enteropathy of the small intestine caused by the consumption of gluten in genetically susceptible individuals, in which participation of the adaptive immune response, and also of the innate immune response is observed. Damage of the intestinal mucosa structure and altered permeability is observed in CD patients, which can lead to microbial translocation and systemic inflammation. Soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP) are postulated as markers of intestinal damage and bacterial translocation in inflammatory pathologies similar to CD. The aim of this study was to evaluate serum levels of sCD14 and LBP in untreated CD patients (active) or CD patients on gluten free diet (GFD) and compare levels with a control group.

Methods and Results: Circulating levels of sCD14 and LBP were determined in 73 adult patients with biopsy proven, 24 of these patients were untreated (active) and 49 on GFD; 55 healthy volunteers were included as controls. Patients were recruited in Instituto de Investigaciones en Ciencias de la Salud and in Fundación Paraguaya de Celíacos, Asunción, Paraguay. The study was approved by the Ethic Committee, IICS and patients provided a written informed consent to participate. Patients on GFD were nutritionally advised. Serum levels of sCD14 and LBP were evaluated by ELISA assay. Mann-Whitney U-test was used to analyze the data; a value of $p<0.05$ was considered statistically significant. We observed higher serum levels of sCD14 in untreated CD patients (median=1776 ng/mL) compared with controls (median=1573 ng/mL) and patients on GFD (median=1472 ng/mL), with $p=0.0185$ and $p=0.0033$, respectively. No significant differences were observed on LBP concentration among CD patients and healthy controls.

Conclusion: In this population sCD14 levels were elevated in active CD patients compared with levels of CD patients on GFD or control group. Increased levels of sCD14 can be a consequence of altered permeability and microbial translocation, therefore may have a potential use as a marker of intestinal inflammation in CD.

Mycobacterium tuberculosis promotes IL-21 and IL-21R expression in alveolar macrophages under in vitro infection

Marco Polo Peralta[1], Marco Polo Peralta[2], Dulce Adriana Mata[2], Brenda Brenda Marquina[2], Jorge Alberto Barrios-Payan[2], Rogelio Hernandez Pando[2]

[1]Instituto Politécnico Nacional, Mexico City, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
marcopolo_308115163@comunidad.unam.mx; marcopolo_308115163@comunidad.unam.mx; dulmat@yahoo.com.mx; brenda.marquina@gmail.com; qcjbp77@yahoo.com.mx; rhdezpando@hotmail.com

Keywords: Macrophages, in vitro, mouse models, IL-21, Pulmonar tuberculosis

IL-21 is a cytokine that was characterized for the first time in 2000. It binds to its receptor (IL-21R), which needs to be coupled to gamma chain to activate several intracellular signals. It´s functions have been linked to proliferation, differentiation and redirection of immune responses in distinct entities, such as intracellular infectious diseases. In the case of Pulmonary Tuberculosis (TB), its presence and production has been related to a protective function, as a consequence of TH1 profile activation with a highly production of TNF-a and IFN-g from TCD-8 lymphocytes as in NK cells as well. However, until now the production of this cytokine has only been documented in TFH (follicular) lymphocytes, TH17, and NKT cells. In a model of progressive pulmonary tuberculosis in BALB /c mice infected with prototype strain H37Rv, we have observed an increase in the expression of IL-21 and its receptor by q-PCR and immunohistochemistry in lung tissue at days 21 and 28 days' post- infection and a reduction on days 60 and 120, which is similar to Th1 cytokine kinetics that is protective against Mycobacterium tuberculosis. Furthermore, the same response was seen in vitro infecting murine alveolar macrophages (MHS) with prototype strain H37Rv or incubating them with Muramyl dipeptide (MDP). Thus, we show for the first-time the IL-21 expression in macrophages infected with mycobacteria suggesting that protection in this intracellular infection is mediated by multiple innate immunity mechanisms including IL-21 production.

Brucella abortus induce mast cells activation in vitro

Gloria Mariana Rodríguez López[1], Marcia Campillo-Navarro[2], Rodolfo Soria-Castro[1], Octavio Rodríguez-Cortés[3], Rubén López-Santiago[1], Sergio Estrada-Parra[1], Iris Estrada-García[1], Raúl Flores-Mejía[3], Rommel Chacón-Salinas[1], Rommel Chacón-Salinas[4]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

[3]Escuela Superior de Medicina (IPN), Mexico City, Mexico

[4]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, Mexico

gloria0012@hotmail.com; marcy265@hotmail.com; rodolfo.soria.castro@gmail.com; octaviordzc@yahoo.com.mx; rlopezsantiago@hotmail.com; sestradap07@hotmail.com; iestrada5@hotmail.com; biodino@hotmail.com; rommelchacons@yahoo.com.mx

Keywords: Brucella abortus, Cytokines, Infection, mast cell, intracellular pathogen

Mast cells play an important role in the immune response to bacterial infections through the release of chemotactic compounds that promote the fast recruitment of immune cells at the site of infection, thus contributing to the elimination of pathogens. Brucellosis is one of the most common and major zoonosis worldwide and is caused by Brucella. Brucella is able to establish a replicative niche and survive inside phagocytic cells, causing chronic disease in animals and humans. Although the role of mast cells in bacterial infections is well established, little is known during Brucella infection. Here we demonstrate the in vitro infection of mast cells by Brucella abortus RB51 and 2308. Mast cells were unable to control the infection, because we observed an increase in colony forming units at 9 hours post infection, where 2308 strain had greater values than RB51 at 24 hours. We demonstrated that respiratory burst showed increased between 60–90 minutes post interaction. Mast cells had the ability to induce the production of cytokines and chemokines that are related to pro-inflammatory process, such as TNF, IL-6, RANTES, MIP-1α and MIP1-β. Based on findings from this study, we conclude that mast cells have the ability to activate and respond to Brucella abortus and may be play an important role in the early induction of immune response.

The Recognition of CD43 by Streptococcus gordonii Hsa promotes macrophage activation

Victor Manuel Villalva-Montaño[1], Alvaro Torres-Huerta[1], Yvonne Rosenstein[1,2], Ismael Secundino[1]

[1]National Autonomous University of Mexico, Cuernavaca, Mexico
[2]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
viman_1993@hotmail.com; avery@ibt.unam.mx; yvonne@ibt.unam.mx; isv@ibt.unam.mx

Keywords: Inflammation, Macrophages, Streptococcus gordonii, PAMPs (Pathogen-associated molecular patterns), CD43 sialomucin

Streptococcus gordonii (S. gordonii) is a non-pathogenic Gram-positive bacterium that is part of the microbiota of the human oral cavity and it is involved in the development of dental plaque. Inflamed gums allow the infection of S. gordonii, promoting transient bacteraemia followed by bacterial colonization of the endocardium and inflammed heart valves, causing endocarditis. The mechanisms and protein interactions that favor the pathogenesis of infective endocarditis are not well known. Hsa is a streptococcal colonization factor relevant for the formation of dental plaque. Hsa could interact with CD43, a transmembrane mucin abundantly expressed in hematopoietic lineage cells, except erythrocytes. CD43 participates in the regulation cell-cell interactions, locomotion, differentiation, proliferation and apoptosis of immune cells. To know the biological implications that arise from the Hsa-CD43 interaction, we used human and mouse macrophages and a mouse model. We found that the recognition of CD43 by Hsa promotes the expression of costimulatory molecules, secretion of cytokines and specific activation of transcriptional factors. Our results suggest that Hsa binding to CD43 in macrophages results in activation signals that may define the outcome of the infection. This interaction may be an initial step of bacterial binding in infective endocarditis due to oral streptococci and underscores the role of CD43 in pathogen recognition, and identifies new therapeutic targets.

Prokaryotic RNA activates endothelial cells and modulates their interaction with human neutrophils

Nahuel Rodriguez Rodrigues, Luis A. Castillo, Daiana Martire Greco, Federico Birnberg-Weiss, Veronica Landoni, Gabriela Cristina Fernandez

Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina

nahuelrodriguezr@gmail.com; luiscastillo718@gmail.com; daianamartire@hotmail.com; federicobirnberg@hotmail.com; vilandoni@yahoo.com.ar; gfernandez@hematologia.anm.edu.ar

Keywords: Endothelial Cells, HUVEC, PMN, HMEC-1, prokaryotic RNA

Polymorphonuclear neutrophils (PMN) are the first cellular line of antibacterial host defense. Previous results obtained in our laboratory show that live but not dead bacteria are able to induce a strong PMN responses, suggesting that bacterial determinants associated with viability are necessary to trigger PMN activation. In this regard, we have demonstrated that prokaryotic RNA (pRNA), present only in live bacteria, is capable of triggering PMN activation and the generation of bactericidal responses, such as the generation of reactive oxygen species and NETs formation. Because PMN adhesion and migration is tightly regulated by endothelial cells through the increase in the expression of adhesion molecules and the secretion of chemokines, in this work our objective was to determine if pRNA can directly activate the endothelium and modulate the interaction with PMN.

The ability of pRNA to activate the endothelium was evaluated using a human microvascular endothelial cell line (HMEC-1), or a primary culture of human umbilical vein endothelial cells (HUVEC). In all experiments, both endothelial cells were stimulated with RNA (1 μg/ml) extracted from E. coli (pRNA) or from human eukaryotic mononuclear cells (eRNA), or left untreated, for 24 hs. The expression of ICAM-1 (a surface glycoprotein that binds to the integrin CD11b present in PMN), measured as the mean fluorescence intensity (MFI) by flow cytometry was increased in pRNA-treated cells compared to the other groups ($p<0.001$). Moreover, the secretion of IL-8 after pRNA exposure was higher ($p<0.05$) measured by ELISA.

To determine the effect on PMN, stimulated HMEC-1 or HUVEC free supernatants were incubated for 30 min. with PMN isolated from healthy donors. Both, cell size (% of high FSC) and CD11b expression (MFI) measured by flow cytometry were increased in PMN incubated with cell free supernatants obtained from pRNA-treated endothelial

cells ($p<0.05$). Through the use of a chemotaxis Boyden chamber, a significant migration of PMN was observed only when supernatants from HMEC-1 that had been pre-treated with pRNA were used as the chemotactic stimuli ($p<0.05$).

We also evaluated the adhesion of PMN to HMEC-1 or HUVEC treated cells, by measuring the activity of PMN alkaline phosphatase with the substrate p-nitrophenylphosphate in the adhered PMN after extensive washing. Higher adhesion was observed only when endothelial cells were pre-treated with pRNA ($p<0.05$ for HUVEC, $p<0.001$ for HMEC-1).

Our results indicate that the presence of pRNA directly activates the endothelium, moreover we also demonstrated that the response of the endothelium to pRNA favors the recruitment and extravasation of PMN.

Down regulation of IL-1β secretion by TGF-β1 in macrophages infected with virus dengue

Brenda Ramírez-Aguero[1], José-Luis Montiel-Hernandez[1], Humberto Lanz-Mendoza[2], Judith Gonzalez Christen[2]

[1]*Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico*
[2]*Centro de Investigación Sobre Enfermedades Infecciosas (CISEI), Cuernavaca, Mexico*
aguerobr@gmail.com; jlmontiel@uaem.mx; humberto@insp.mx; judith.gonzalez@uaem.mx

Keywords: Dengue, Inflammation, macrophage, IL-1β, TGF-β1

Introduction: Infection with dengue virus (DENV) can occur with a broad spectrum of manifestations, ranging from asymptomatic infections to mild, if it is present with fever, headache, myalgia, rash and muscular pain, or severe if in addition to a series of manifestations there is loss of vascular permeability and plasma leakage, with risk of loss of organic function (1). Understanding the pathogenic mechanisms for DENV is complicated, because it involves viral cytotoxicity, underlying host genetics and comorbidities such as diabetes and hypertension (2). A proposal associates the severity of the disease with the development of an uncontrolled immune response, with an increase in cytokines such as TNF, IL-1β, IL-8, IL-6 and chemokines that damage human microvascular endothelial barrier (3,4), and also with the disturbance in the balance of pro or anti- inflammatory cytokines, as IL-10 and TGF-β (5, 6). In our laboratory we had observed that patients with severe disease have higher serum IL-1β concentration, and in vitro infected human macrophages secrete TGF-β1 later than TNF and IL-1β. TGF-β1 performs diverse cellular functions, including regulation of secretion of inflammatory molecules. Different studies with other systems have demonstrated that there is a negative relationship between TGF-β and IL-1β, because the final proteins produced by the signaling of TGF-β -Smad6/7- induced negative regulation of IL-1R/TLR signaling (7). In macrophages this factor induces a M2-like phenotype characterized by up-regulation of the anti-inflammatory cytokine IL-10, and down-regulation of the pro-inflammatory cytokines (8). There are few studies in vitro that have evaluated the role of TGF-β in dengue infection and its relation to the inflammatory process, so it is unknown whether they are related.

Aim: To evaluate the ability of TGF-β1 to inhibit the secretion of IL-1β in macrophages infected by DENV.

Methods: THP-1 cells differentiated with 10 nM PMA were used in for infection with Dengue Virus 2 Nueva Guinea for 24h, with a MOI=1. LPS (E. coli 011:B4 SIGMA) was used at 10 ng/mL and human TGF-β1 (Peprotech) at 20ng/mL. Infection was evaluated by fluorescent microscopy (AbCAM anti Den-2) and IL-1β secretion in supernatant was quantified by ELISA kit (Bioresearch). Cells were treated with PBS, LPS or DENV to compare with those cells pre-incubated with TGF-β1 for 3 hours before LPS or DENV, or 2 hours post stimulated with LPS or DENV.

Results: After 24 h of infection, 40% of cells were positives for Dengue infection, determinate by microscopy, and confirmed by PCR. To confirm that TGF-β1 is active in these cells, we demonstrate the phosphorylation of SMAD 2/3 by Western blot. At this time cells stimulated with LPS and DENV infected cells secreted 18 and 1 pg/ml of IL-1β, respectively, but in cell pre-incubated with TGF-β1 previously to treatment did not produced this cytokine. In the assay where the cells were first stimulated with LPS or were infected with DENV, and subsequently added TGF-β1, it was observed that the change in the secretion of IL-1β between cells with or without treatment was lesser of 25%.

Conclusions: TGF-β1 abrogates the secretion of IL-1β in macrophages only when is present previously before the infection with DENV, but not in infected cells.

REFERENCES

1) Jaenisch, T et al. BMC Infect Dis. 2016; 16: 120
2) Yacoub S, Mongkolsapaya J, Screaton G. Curr Opin Infect Dis. 2013; 26:284-9
3) Soe HJ, et al. J Gen Virol. 2017; 98:2993-3007
4) Srikiatkhachorn A, Mathew A, Rothman AL. Semin Immunopathol. 2017; 39:563-574
5) Iani FC, et al. Am J Trop Med Hyg. 2016; 95:193-200
6) Tsai, TT, et al. J Biomed Sci. 2013; 20:40
7) Lee, YS et al. BBCR 2010; 393: 836–843
8) Zhang, F et al. Oncotarget. 2016; 7:52294-52306

Effect of 30-kDa protein from Salmonella typhimurium in the production of extracellular traps and proinflammatory cytokines by neutrophils

Juan Manuel Agraz Cibrian[1], José Francisco Zambrano Zaragoza[1], Norberto Vibanco Pérez[1], Ma. de Jesus Durán Avelar[1], Jorge Gutiérrez Franco[1], Alejandro Vazquez Reyes[1], Iris Celeste Tovar Ocampo[1], Miriam Fabiola Ayón Pérez[1], Liliana Ortiz Martínez[2], Dalia Verónica Vela Flóres[1], Brallan Antonio Mejía Macias[1], Osmar Miguel Gutiérrez[1]

[1]Universidad Autónoma de Nayarit, Tepic, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
jumaagci@gmail.com; jzambran@uan.edu.mx; novipe@hotmail.com; mduran@uan.edu.mx; jorge.gutierrez@uan.edu.mx; avazquez@uan.edu.mx; icto.qfb@gmail.com; miry_03@hotmail.com; lyn_gdl@hotmail.com; zofre_kya@hotmail.com; bamm_9408@hotmail.com; osmargutierrezc@hotmail.com

Keywords: Cytokines, neutrophil extracellular traps, ankylosing spondylitis, Inflammatory Response, 30-kDa protein

Ankylosing spondylitis (AS) is an autoimmune disease. Although the etiology of autoimmunity is unknown, the involvement of genetic and environmental factors, such as HLA-B27 and bacterial infections, has been described. In previous works our group has reported that a 30 kDa protein of Salmonella typhimurium is associated with AS, because patients with AS have higher levels of serum IgG3 anti-p30 than healthy controls. Additionally, the presence of DNA from Salmonella sp. in the synovial fluid of patients with spondyloarthritis have been reported, and the intestinal disbiosis has been proposed as one of the main factors involved in the immunophatogenesis of AS, because an alteration in the microbiota allows to bacterial or their products to across the intestinal barrier, triggering the activation of the immune system, the innate and adaptive, supporting the involvement of bacterial antigens in AS. On the other hand, AS is an inflammatory disease, in which the main cell involved are neutrophils, the first line of defense of innate immunity. In addition to phagocytosis, degranulation and secretion of cytokines, the most recent of the microbicidal mechanism involved in pathogens elimination of neutrohpils is known as NETs, although their overproduction may trigger tissue damage and induce inflammation. Considering these data, in this work we try to determine if there is a relationship between the anti-p30 antibodies, the induction of NETs and secretion of proinflammatory cytokines in patients with AS.

Objective: To determine the effect of p30 alone or in immune complex in the production of NETs and release of cytokines by neutrophils.

Methodology: Neutrophils were obtained from healthy subjects and stimulated with p30 from Salmonella typhimurium alone or in immune complex (with the sera of patients with AS). The production of NETs was observed by fluorescence microscopy and the concentration of cytokines (IL-6, IL-1, TNF-, IL-12, IL-8 and IL-10) was determined by CBA.

Preliminary Results: Our results show that the percentage of NETs in neutrophils stimulated with p30 in combination with serum from patients with AS was higher (70.52 ± 16.24) compared to those stimulated with PMA [39.78 ± 14.50; (p = 0.0190)] or with PBS (negative control) [9.94 ± 12.12; (p = 0.0095)]. Furthermore, no NETs were observed when neutrophils were stimulated with p30 alone or p30 with sera from healthy control. On the other hand, no statistical differences in the concentration of cytokines secreted by neutrophils activated with the different stimulus were found, although the highest concentration of IL-6, IL-1 and IL-8 were found in neutrophils stimulated with p30 in combination with serum of patient with AS or healthy controls.

Conclusion: In this study we suggest that the p30 in immune complex with sera from patients with AS could be involved in the pathological mechanisms in AS, by contributing to the maintenance of the inflammatory environment through the higher amounts of NETs and the induction of proinflammatory cytokines.

Taenia crassiceps infection induces the recruitment of low density neutrophils with suppressive activity

Cuauhtemoc Angel Sanchez Barrera, Yadira Ledesma Soto, Sandy Reyes Martinez, Yolanda Gonzalez Hernandez, Blanca Estela Callejas Piña, Ilse Chavez Soto, Guillermo Alvarado Hernandez, Luis Ignacio Terrazas

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
angel.casb@hotmail.com; lesy_790413@yahoo.com.mx; sandy_reyes25@comunidad.unam.mx; yolandagonzalezhdz@gmail.com; aletse_bianca@hotmail.com; ilsebios01@gmail.com; galvaradohernandez@gmail.com; literrazas@campus.iztacala.unam.mx

Keywords: PDL-1, Taenia crassiceps, PDL-2, Low density neutrophils, High Density Neutrophils

Neutrophils are the most abundant population of leucocytes in peripheral blood, they constitute between 50–70% of total white blood cells. It has been largely known that neutrophils are the first cells to be recruited during infection and play a key role in the resolution of infections, also these cells are known to be associated with diseases that involve an exacerbated inflammatory response. However, in recent years the activity and function of neutrophils has been more carefully analyzed and now is known that neutrophils also participate in the resolution of inflammation and the maintenance of homeostasis. Even two relatively new subpopulations have been described such as neutrophils called N1 or High Density Neutrophils (HDN) with antitumoral activity and N2 or Low Density Neutrophils (LDN) with pro-tumoral activity. Besides, other studies reported that neutrophils may have anti-inflammatory activity through the release of different cytokines or the expression of some suppressive molecules, such as Programed Death Ligand 1 (PDL-1). Taenia crassiceps is a helminth that is able to modulate the immune response in its hosts by inducing polarized Th2-type responses and recruiting alternatively activated macrophage that are associated with an anti-inflammatory microenvironment. Interestigly the role of neutrophils in this infection and in general helminth infections is poorly known. Therefore, the goal of this study was to evaluate possible phenotypic and functional differences in neutrophils during the infection with T. crassiceps.

BALB/c female mice were infected with 20 cysticerci of T. crassiceps or tioglycollate-induced sterile peritonitis. Two days, two weeks, four or eight weeks following infection, peritoneal exudate cells (PECs) were collected to analyze the recruitment of neutrophils at different times after infection. At the same times, by flow cytometry, we analyzed the expression of molecules such as PDL-1, PDL-2 , as well as the

production of reactive oxygen species (ROS) in neutrophils. To do that we separate LDN by a density gradient and then isolated Ly6Clow Ly6G+ cells by sorting and culture them total splenocytes previously stimulated with anti-CD3, in a 4:1 (splenocytes: Ly6Clow Ly6G+) proportion. Then we evaluated mRNA expression for TNF-α and TGF-β. We found that 7.02% of PECs were neutrophils at two days after infection and this percentage was significantly reduced at two and four weeks of infection. Interestingly at the eight weeks post-infection the neutrophil percentage increased at 14%. When the expression of PDL-1 in neutrophils was analyzed, we found that those neutrophils recruited by sterile peritonitis expressed lower levels of PDL-1 (30%) than the neutrophils recruited at both acute infection (two days) of infection which reach 79% and chronic infection (59%). Remarkably, we found that only the neutrophils recruited at eight weeks post-infection expressed PDL-2 in a 23%. Moreover, we demonstrated that a less percentage of neutrophils produced ROS at the eight weeks of infection (86 %) compared with two days of infection (95%) and sterile peritonitis (98%). On the other hand, neutrophils were isolated by a density gradient and we confirm that the LDN from the eighth week of infection expressed more PDL-1, PDL-2 and produced less ROS. However, when we analyzed the purity of the sample, we found that only about 20 % were neutrophils. For this reason, we decided to isolate the Ly6Clow Ly6G+ cells fraction by flow cytometry cell sorting from the LDN fraction. With this method, we obtained 97 % of purity and found cells with Granulocytic Myeloid Derived Suppressor Cells and mature neutrophil morphology. These Ly6Clow Ly6G+ isolated cells from de chronic infection were able to suppress the proliferation of splenocytes in contrast with the Ly6Clow Ly6G+ cells from the sterile peritonitis. Also, these chronic infection Ly6Clow Ly6G+ expressed more mRNA of TGF-β a reported cytokine produced by N2 neutrophils, while, Ly6Clow Ly6G+ from sterile peritonitis expressed more mRNA of TNF-α a pro-inflammatory cytokine. In conclusion we reported that the infection with the helminth Taenia crassiceps induces the recruitment of neutrophils with a differential expression of PDL-1, expression of PDL-2 and suppressive capacity, only in the chronic infection in contrast to the neutrophils recruited by sterile peritonitis.

Polyacrylic acid-coated iron oxide nanoparticles could be usefully tool to in vivo track mononuclear phagocytes subsets without extensively compromise most of their basic functions

Manuela María Giraldo, Jeaneth Patricia Urquijo, Melissa Trejos-Ceballos, Oscar Luis Arnache-Olmos, Mauricio Rojas

Universidad de Antioquía, Medellín, Colombia
mgiralvi@gmail.com; jpatriciaurquijo@gmail.com; iris_019@hotmail.com; oscar.arnache@udea.edu.co; mauricio.rojas@udea.edu.co

Keywords: Macrophages, Monocytes, Nanoparticles, T cells, magnetic iron particles

Monocytes play critical roles in different inflammatory processes. They promote the inflammation, the regulatory responses and tissue remodeling. In very diverse pathologies, the alterations in the balance of these monocyte responses can disrupt and trigger immunopathological processes. Their characterization in these processes requires procedures that in general are very invasive and cannot be repeated very frequently without exposing patients to greater risks. Therefore, trying to generate non-invasive strategies to detect monocytes in vivo, we have screened in vitro different iron derivatives with supermagnetic properties within which the nanoparticles coated with acrylate coating graze to be the most promising.

To further determine if the polyacrylic acid-coated iron oxide nanoparticles (PACIONP) may specifically interact with blood cells, total human blood was exposed to PACINP at 37°C during 1 h and the changes in the cell side scattering were evaluated by flow cytometry (BD LSRFortessa™) after washing out the excess of PACIONP. Only CD14++ CD16- and CD14++ CD16positive monocytes registered and increased in the side scattering, and there were not changes in granulocytes and lymphocytes.

To verify the specificity of this interaction, monocytes were highly purified by the electromagnetic cell sorting (MOFLO XDP) into the two major monocyte subsets and exposed to PACIONP at 37°C during 1 h. Then the free nanoparticles were removed by extensively washing and the cells were hydrolyzed with nitric acid and the total amount of iron was determined by atomic mass spectrometry. These assays clearly indicated that although both monocytes uptake the PACIONP, CD14++ CD16positive (non-classical monocytes)(1) presented ten times higher amounts of iron due to the major uptake of the PACIONP.

Thus, we can certainly affirm that the PACIONP specifically interact with monocytes rather than with any other phagocytes, launching them as potential tool to track monocytes in vivo. Nevertheless, prior to their use in vivo we decide to in vitro screen several functions and putative effects on human mononuclear phagocytes and macrophages previously differentiated in the presence of the PACIONP.

When fresh isolated monocytes were differentiated into macrophages (MDM)(2) in the presence of the highest concentration of PACIONP that do not compromise the cell viability, we evaluate different parameters associated with monocyte differentiation and function. The PACIONP did not altered the expression of CD80, CD86, CD206, HLA-DR and did not affected the activity of the non-specific esterase compared with untreated controls (1, 3, 4). Nevertheless, there was a reduction in the expression of CD36 in monocytes differentiated into macrophages in the presence of the PACIONP.

Since, the in vivo tracking of tissue monocytes by PACIONP should require the exposure to magnetic fields, mononuclear cells were treated with PACIONP and exposed to a constant magnetic field with 1,5 T during 10 min. Then, we evaluated for the changes in the mitochondrial permeability status, the DNA damage, the cleavage of PARP and the phosphorylation of the H2AX histone and the incorporation of BrdU by flow cytometry were evaluated in total mononuclear cells. We found that cells treated with PACIONP and exposed to the magnetic (1.5 T/10 min) did not undergo cell membrane damage, loss of mitochondrial membrane potential, DNA damage or increased in the phosphorylation of H2AX.

In addition, we also evaluate if the differentiation in the presence of PACIONP could compromise their capability to activate T cells. To this, MDMs from tetanus vaccinated people were differentiated in the presence of PACIONP during 120 h. Then highly purified autologous CD3+ cells by electromagnetic cell sorting were stained with CFSE, added to monocytes and then stimulated with tetanus toxoid or PHA during additional 96 h. Cytokines were evaluated before adding T cells and after before the determination of T cell proliferation(5).

Monocyte-derived macrophages, differentiated in the presence of PACIONP and with a transient magnetic field exposure, did no loss their capacity to induce proliferation of T lymphocytes in response to antigens. In addition, although there was no effect in the cytokine accumulation before the addition of T cells due to PACIONP or the magnetic field, at the end the co-culture, only the presence of PACIONP, but not the exposure to the magnetic field, had an increase in the accumulation of IL-10 and IL-6.

We conclude that the PACIONP could be a putative tool, maybe as contrast agent, to track monocytes in vivo, and more interestingly to differentially track monocyte subsets. PACIONP did not affect phagocyte viability or function, which allows us to attribute a good degree of biocompatibility and therefore. Although the way by which in the co-culture we observed higher accumulation of IL-10 and IL-6 it was not established, this should be an interesting point to further consider to prevent putative side effects after in vivo exposure to this nanoparticles.

REFERENCES

1. D. Castano, L. F. Garcia and M. Rojas: Increased frequency and cell death of CD16+ monocytes with Mycobacterium tuberculosis infection. Tuberculosis (Edinb), 91(5), 348-60 (2011) doi:10.1016/j.tube.2011.04.002
2. M. Hristov, S. Schmitz, F. Nauwelaers and C. Weber: A flow cytometric protocol for enumeration of endothelial progenitor cells and monocyte subsets in human blood. J Immunol Methods, 381(1-2), 9-13 (2012) doi:10.1016/j.jim.2012.04.003
3. D. Castano, L. F. Garcia and M. Rojas: Differentiation of human mononuclear phagocytes increases their innate response to Mycobacterium tuberculosis infection. Tuberculosis (Edinb), 94(3), 207-18 (2014) doi:10.1016/j.tube.2014.01.001
4. D. Castano, L. F. Barrera and M. Rojas: Mycobacterium tuberculosis alters the differentiation of monocytes into macrophages in vitro. Cell Immunol, 268(2), 60-7 (2011) doi:10.1016/j.cellimm.2011.02.006
5. E. B. Cook, J. L. Stahl, L. Lowe, R. Chen, E. Morgan, J. Wilson, R. Varro, A. Chan, F. M. Graziano and N. P. Barney: Simultaneous measurement of six cytokines in a single sample of human tears using microparticle-based flow cytometry: allergics vs. non-allergics. J Immunol Methods, 254(1-2), 109-18 (2001)

Valproic acid inhibits the production of gamma-interferon by murine NK cells

Rodolfo Soria-Castro[1], Gloria Rodríguez-López[1], Marcia Campillo-Navarro[2], Alma D. Chávez-Blanco[3], Raúl Flores-Mejía[4], Sergio Estrada-Parra[1], Iris Estrada-Garcia[1], Jeanet Serafin-López[1], Rommel Chacón-Salinas[1,5]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
[2]Departamento de Fisiología y Farmacología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico, Mexico
[3]Instituto Nacional de Cancerología (INCan), Tlalpan, Mexico
[4]Escuela Superior de Medicina (IPN), Mexico City, Mexico
[5]Unidad de Desarrollo e Investigación en Bioprocesos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
rodolfo.soria.castro@gmail.com; gloria0012@hotmail.com; marcy265@hotmail.com; celular_alma@hotmail.com; biodino@hotmail.com; sestradap07@hotmail.com; iestrada5@hotmail.com; jeaserafin@hotmail.com; rommelchacons@yahoo.com.mx

Keywords: Listeria monocytogenes, NK cells, in vitro, mouse models, gamma interferon, IL-18, IL-12p70, splenocytes, valproic acid (VPA)

NK cells are one of the main cells in response to viral and bacterial infections. These cells are important mediators of the innate immune response, either by exerting cytotoxic activity or by producing gamma-interferon (IFN-g). For instance, during L. monocytogenes infection IL-12 and IL-18 produced by macrophages and dendritic cells activates NK cells to produce large amounts of IFN-g, which is crucial for the activation of macrophages and dendritic cells, leading to an increase in the production of reactive oxygen and nitrogen species, MHC class II, chemokines and inducing autophagy, which are associated with an early control of bacterial infection. Valproic acid (VPA) is the drug of choice to control seizures in epileptic patients and for treatment of other neurological diseases. Moreover, recent evidence indicates that VPA has immunomodulatory properties that are exploited in the therapy against cancer. During infections, the effects exerted by VPA are contradictory, ranging from exacerbation to protection of infection with bacteria, virus and fungi.

The goal of this study was to analyze the effect of VPA on the production of IFN-g by NK cells during L. monocytogenes infection. We observed that VPA inhibited purified murine NK cells to produce IFN-g after stimulation with IL-12 and IL-18. Moreover, VPA drastically reduced the levels of IFN-g in the supernatants of splenocytes

infected with L. monocytogenes. Finally, we found that VPA strongly impacts the number of NK cells producing IFN-g from the culture of splenocytes infected with L. monocytogenes. These results show that VPA interferes with NK activation during L. monocytogenes infection.

The microRNA miR-671-5p targets genes involved in antigen processing and presentation in porcine monocytes stimulated with probiotic BB12

Marlene Bravo-Parra, Veronica Mata Haro

Center for Research in Food and Development (CIAD), Hermosillo, Mexico
marlene.bravo@estudiantes.ciad.mx; vmata@ciad.mx

Keywords: Immunomodulation, Probiotics, Microarray, monocyte, post-transcriptional regulation, porcine microRNAs, antigen processing and presentation pathway

Introduction: The antigen presenting cells are able to differentiate between pathogenic microorganisms and those as commensal or probiotics; nevertheless, still remains unclear why only some commensal microorganisms can be used as probiotics and have beneficial effect on human and animal health, since they share many structural features. A novel research attempt to explain these molecular mechanisms is the modulation of immune response mediated by microRNAs (miRNAs). The objective of this work is to identify target genes of miRNAs that were differentially expressed in porcine monocytes stimulated with the probiotic Bifidobacterium animalis subp. Lactis BB12 (BB12), involved in exogenous antigen processing and presentation.

Material and Methods: Peripheral blood monocytes from healthy pigs from four to six weeks-old were used. The monocytes were stimulated in 1:100 ratio (monocytes:bacteria) for six hours and unstimulated cells were used as control. After that time, RNA was extracted and performed a miRNA microarray. To perform the prediction analysis, the miRNAs that were differentially expressed with a value of $p<0.05$ and media fluorescence signal >500 in t-test for two independent samples between control and stimulated monocytes with BB12 of microarray were chosen. The Ensembl bioinformatic tool (Sus scrofa 11.1) was employed to obtain the sequences of porcine 3'UTR regions of the genes of interest. The mature sequences of miRNAs were provided from microarray. Additionally RNAHydrid was used to predict target genes involved in antigen processing and presentation pathway.

Results: Twenty-five miRNAs were selected, of those, 16 resulted overexpressed after BB12 stimulation, while nine were underexpressed. The minimal free energies (mfe) values of the hybridization reactions between miRNAs and 3'UTR regions sequences of genes ranking between -13.7 a -42.3 kcal/mol. We selected 198 reactions that obtained

an mfe≤-25 to -42.3 kcal/mol. Twenty-three miRNAs were bound to the transcripts of MHC II pathway. Of these, only three got a value of mfe≤-40 kcal/mol. In this prediction, miR-671-5p stands out for its complementarity to the 3'UTR regions transcripts of CD74, RFXANK and RFX5 with values of mfe≤-40 kcal/mol. In the microarray miRNA miR-671-5p was over expressed with respect to control, hence the overexpression of this miRNA could affect the translation of genes that participate constructing and carrying the MHC II molecule before being presented. Likewise, miR-4334-3p was mostly differentiated in the present experiment, which was found underexpressed with respect to control. Others miRNAs also resulted with differences, like miR-9802-3p and miR-9858-5p; however, no information on human targets is available at present. In this analysis miR-4334-3p binds to the 3'UTR of RFXANK (-34.9 kcal/mol), DC4 (-32.9 kcal/mol) and SLA-DRB1 (-32.4 kcal/mol) transcripts respectively, that are molecules also involved in antigenic presentation.

Conclusion: The probiotic BB12 induced expression of miRNAs capable of binding important genes involved in constructing and carrying the MHC II molecule. Nevertheless, this information remains to be validates by RT-qPCR assay.

NMDA Receptor antagonist ketamine induces a regulatory macrophage program on human monocytes

Wanda Nowak[1], Ivana G Estecho[1], Federico M Daray[1], Eugenio A Carrera Silva[2], Andrea E Errasti[1]

[1]*Universidad de Buenos Aires, Buenos Aires, Argentina*
[2]*Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina*
wannowak@yahoo.com; ivanaestecho@gmail.com; federicodaray@gmail.com; carrerasilva@yahoo.com.ar; andreaerrasti@gmail.com

Keywords: Depression, Ketamine, NMDA receptor, mTOR pathway, Macrophage polarization

Introduction: The NMDA antagonist, ketamine, discovered more than 50 years ago, is still used as an anesthetic, but recently a new indication emerged for this old drug as a fast and potent antidepressant (Potter and Choudhury, 2014). The biology of this effect is poorly understood but new research implicates that one of the main targetsof its action is the immune response (Laudanski et al., 2015; Kiraly et al., 2017). Strong evidence demonstrates that inflammation plays an important role in the pathophysiology of depression (Roman et al., 2013; Haapakoski et al., 2016; Mechawar and Savitz, 2016). In the same sense, macrophages are key innate players cells promoting inflammation or regulatory signals. This balance depends on the environmental stimuli as well as the cytokine milieu. Pro-inflammatory (M1) program is induced by inflammatory stimuli such as INFγ, while an anti-inflammatory (M2) profile could be induced by type-2 cytokines such as IL-4 and IL-10 (Mantovani and Locati, 2009; Gordon S., 2003).

The aim of the present study was to elucidate the effect of ketamine on human macrophage differentiation and polarization, the type of receptor involved, and the gene program elicited by this drug.

Methods: 10–15 ml of blood was drawn from healthy volunteers and mononuclear cellular fraction was obtained by Ficoll gradient centrifugation. CD14+ monocytes were isolated using a positive selection kit (Stemcell) and 1.5 x 105 cells were seeded in 48-well plates with 10% FBS supplemented RPMI medium. After 7 days of culture, cells were harvested for phenotype characterization and gene expression analysis.

Ketamine (0.1, 1 and 10 μM) was added after 6 days of macrophage differentiation and stimulated with LPS (1 ng/ml) to analyze activation markers and cytokine production by flow cytometry and ELISA, respectively. On the other hand, ketamine (0.1, 1 and 10 μM) was added from day 0 of CD14+ cell culture and differentiation and polarization markers were analyzed. Also, in independent experiments, MK-801 (10 μM, an NMDA receptor selective antagonist) or NBQX (10 μM, an AMPA receptor selective antagonist) were added from day 0.

Gene expression analysis to discriminate pro-inflammatory and regulatory macrophages was performed by qPCR.

LPS (1 ng/ml) + INFγ (50 ng/ml), IL-4 (40 ng/ml) or dexamethasone (0.1μM) were used as positive controls for macrophage polarization.

Results: Ketamine treatment, 30 minutes before the addition of LPS on day 6, dampens macrophage activation determined by lower expression of CD80 and HLADR. Moreover, when ketamine was added to monocyte cultures from day 0 and then stimulated overnight with LPS at day 6, expression of CD80 and HLADR as well as TNF-α production was also significantly reduced.

Ketamine treatment of CD14+ monocytes from day 0 of culture strongly induced the expression of CD163 and MERTK with intermediate levels of CD64, compatible with a regulatory M2c-like phenotype.

The NMDA receptor antagonist, MK-801, induced a similar macrophage phenotype as ketamine in contrast to the AMPA receptor antagonist, NBQX.

Gene expression analysis demonstrate that ketamine up-regulates mRNA levels of TGM2 and CCL22, genes related to an M2 profile. Quite the opposite, ketamine had no effect on mRNA levels of CXCL10, a gene related to an M1 profile. Interestingly, ketamine promoted the up-regulation of mRNA levels of mTOR pathway related genes, SGK1 and eIF4B.

Conclusions: The present study demonstrates that ketamine dampens the acute inflammatory response elicited by LPS on differentiated macrophages as well as induces a regulatory program on human monocytes, promoting an M2c-like phenotype in these cells. Moreover, this regulatory program appears to be mediated by the NMDA receptor. Furthermore, ketamine induces a particular gene expression profile involving components of the mTOR pathway.

REFERENCES

Gordon, S. (2003) Alternative activation of macrophages. *Nature Reviews in Immunology* 3:23-35.

Haapakoski, R, Ebmeier, KP, et al. (2016) Innate and adaptive immunity in the development of depression: An update on current knowledge and technological advances. *Progress in Neuro-psychopharmacology and Biological Psychiatry* 66:63-72.

Kiraly DD, Horn SR, Van Dam NT, et al. (2017) Altered peripheral immune profiles in treatment-resistant depression: response to ketamine and prediction of treatment outcome. *Translational Psychiatry* 7, e1065.

Laudanski, K, Meng Qing, MA, Oszkiel, A, et al. (2015) Ketamine affects in vitro differentiation of monocyte into immature dendritic cells. *Anesthesiology* 123:628-41.

Mantovani, A and Locati, M. (2009) Orchestration of macrophage polarization. *Blood* 114:3135-36.

Mechawar, N. and Savitz J (2016) Neuropathology of mood disorders: do we see the stigmata of inflammation? *Translational Psychiatry* 6(11):e946.

Potter, DE and Choudhury M (2014) Ketamine: repurposing and redefining a multifaceted drug. *Drug Discov Today* 19(12):1848-1854.

Roman, A, Kreiner, G, et al. (2013) Macrophages and depression - a misalliance or well-arranged marriage? *Pharmacological Reports* 65(6):1663-1672.

Immunomodulation as a therapeutic tool for chronic inflammation during HIV infection

Natalia Andrea Taborda[1,2], Aura Maria Gil Villa[1], Manuel Geronimo Feria Garzon[2], Juan Esteban Gomez Bedoya[2], Jorge Lujan Tangarife[2], Juan Carlos Hernández López[2,3], Maria Teresa Rugeles[2]

[1]*Corporación Universitaria Remington, Medellín, Colombia*
[2]*Universidad de Antioquía, Medellín, Colombia*
[3]*Universidad Cooperativa de Colombia, Bogotá, Colombia*
nataliataborda@gmail.com; aura.gil@uniremington.edu.co; geronimoferia23@gmail.com; foxjuanes@gmail.com; lujanarmy@gmail.com; juankhernandez@gmail.com; maria.rugeles@udea.edu.co

Keywords: Inflammasomes, Inflammation, Sulfasalazine, HIV infection, clinical manifestations

Introduction: Inflammation is a physiological process that is elicited to control pathogens invasion and repair affected tissues. This process might become a pathological event when regulatory mechanisms are altered. Some infectious agents, such as the human immunodeficiency virus type 1 (HIV-1) has the ability to exacerbate the inflammatory response, leading to the progressive loss of the functional capacity of the immune system. This phenomenon affects different cellular subpopulations, including monocytes/macrophages and dendritic cells, which are activated by the recognition of microbial products through Toll-like receptors (TLRs) and nucleotide-binding domain (NOD)-like receptors (NLR). This process induces the expression of pro-inflammatory cytokines such as interleukin 1β (IL-1β) and IL-18 that in turn promote the differentiation of naïve CD4+ T lymphocytes towards the main HIV-1 targets, the Th1 and Th17 cells.

Particularly, some pattern recognition receptors (PRR) along with the adaptor protein ASC and pro-caspase-1, assemble into multi-molecular complexes called inflammasomes. These complexes activate the caspase-1, which is responsible for the proteolytic maturation and secretion of the IL-1β and IL-18 cytokines, as well as the pyroptosis, an inflammatory cell death type. Up to date, five inflammasomes have been described and further characterized, including NLRP1, NLRP3, and NLRC4 (belonging to the NLR family), the AIM2 (absent in melanoma 2), and the recently described Pyrin. All of them are activated in response to different stimuli, including infectious agents and changes in cell homeostasis.

We have previously demonstrated that HIV-1-infected individuals who naturally control viral replication, known as HIV-controllers, exhibited lower expression of inflammasome-related genes, such as IL-1β, IL-18 and caspase -1, as well as lower

production of IL-1β through the NLRP1, NLRC4 and AIM2 inflammasomes in gut-associated-lymphoid tissue (GALT) and peripheral blood mononuclear cells (PBMCs), compared to HIV-progressors (submitted to PlosOne). These results suggest that the inflammasomes could be involved in the pathogenesis and disease progression in HIV-1 infected individuals.

Therefore, the search for therapies to modulate this inflammatory process constitutes an important field of research. In this sense, there is the possibility that sulfasalazine (SSZ), an anti-inflammatory compound, previously used empirically, but successfully, for treatment of gut inflammatory diseases in HIV-1-infected patients, is exerting its action by inhibiting inflammasomes. However, this hypothesis requires further exploration.

Objective: To determine the potential inhibitory effect of SSZ on inflammasomes and Toll-like receptors activity, by measuring the IL-1β release and mRNA expression of the inflammasome-related genes in cell cultures of PBMCs and to evaluate if the effect is maintained even in the context of the HIV infection.

Methodology: A total of ten healthy donors (seronegative for HIV-1) were included, as well as a group of ten HIV-progressors (viral load median: 18,736 copies/mL, and CD4+ T-cell counts median: 438 cell/μL) in absence of HAART. The SSZ, analytical standard ≥98%, was dissolved in 1% dimethylsulfoxide. The following inflammasome and TLR agonists were used: Adenosine triphosphate (ATP) for NLRP3; purified flagellin of Salmonella typhimurium (FLA) for NLRC4; Poly(deoxyadenylic-deoxythimidilic) acid (poly(dA;dT)) for AIM2; Muramyl dipeptide (MDP) for NLRP1; Lipopolysaccharide (LPS) B5 from the Gram-negative bacteria E. coli 055: B5 for TLR4; Polyinosinic-polycytidylic acid (poly(I:C)) for TLR3; R848 for TLR7/8; Pam2CSK4 for TLR2; and CpG-K16 for TLR9.

The PBMCs were isolated using Ficoll-hypaque gradient. Then, 1x106 PBMCs were primed for 2 hours with 50 pg/mL of LPS. The second activation signal was induced with the specific concentration of inflammasome agonists: 2 mM of ATP; 500 ng/mL of FLA; 50 μg/mL of poly(dA;dT); and 0.1 μg/mL of Muramyl dipeptide (MDP). Simultaneously, 1 mM of SSZ was added in a final volume of 300 μL. After four hours of incubation, or 2 hours for ATP-treated PBMCs, the supernatants were harvested.

In the TLR assays, 1.5x105 PBMCs were cultured and stimulated with the following TLR agonists: 100 ng/mL LPS; 1 μg/mL poly(I:C); 1 μg/mL R848; 40 ng/mL Pam2CSK4;

and 4 μg/mL CpG-K16. Simultaneously, 1 mM of SSZ was added in a final volume of 300 μL. After 18 hours of incubation, the supernatants were harvested.

The IL-1β detection was performed by ELISA in supernatants of cultured cells. To determine the transcriptional expression of inflammasome-related genes (NLRP3, NLRP1, NLRC4, NLRP6, AIM2, ASC, and caspase-1) and their products (IL-1β and IL-18), total RNA extraction from PBMCs was performed using the RNeasy Mini Kit. RNA was treated with DNAse and the cDNA was synthesized using the RevertAid H Minus First Strand cDNA Synthesis. Gene expression was quantified by real-time PCR, using Maxima SYBR Green/ROX qPCR Master Mix.

To compare the immunomodulatory effect of SSZ, Wilcoxon matched-pairs signed rank one-tailed test was used. A p value <0.05 was considered statistically significant. Correlation analyses were based on Spearman correlation coefficient calculations. The statistical tests were performed using the GraphPad Prism Software version 5.0.

Results: To determine if the SSZ has an immunomodulatory effect on the inflammasomes in uninfected and HIV infected patients, the production of IL-1β was measured in supernatants of PBMCs of both groups of individuals and stimulated with the inflammasomes agonists in presence or absence of SSZ. As previously reported, treatment with the ATP, MDP, poly(dA;dT) and FLA induce the production of IL-1β. Interestingly, The IL-1 β release was significantly decreased in the presence of SSZ, in both uninfected and infected individuals. Additionally, a preliminary exploration of the effect of SSZ on TLRs showed that this compound induced a significant reduction in IL-1β production in response to stimulation with specific agonists of TLR2, TLR3, TLR4, TLR7/8 and TLR9.

Conclusions: These preliminary results suggest that SSZ, even in HIV infected patients with clinical progression, could modulate the activity of inflammasomes and the signals required for their activation, such as those associated with TLRs. Additional studies should determine the potential useful as a short-treatment strategy to control clinical inflammatory manifestations during the HIV infection.

Lactobacillus paracasei fermented milks: immunomodulatory properties of the nonbacterial fraction on gastric and intestinal epithelial cells

Ana Agustina Bengoa[1], Agustina Errea[2], Carolina Iraporda[3], Martin Rumbo[2], Analia Graciela Abraham[1,4], Graciela Liliana Garrote[1]

[1]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), La Plata, Argentina
[2]Instituto de Estudios Inmunológicos y Fisopatológicos (IIFP, UNLP-CONICET), La Plata, Argentina
[3]Facultad de Ingeniería, National University of Central Buenos Aires, Olavarría, Argentina
[4]Área Bioquímica y Control de Alimentos, Facultad de Ciencias Exactas, UNLP, La Plata, Argentina
bengoaagustina@gmail.com; aquinohaynadie2@gmail.com; carolinairaporda@gmail.com; rumbo.martin@gmail.com; aga@biol.unlp.edu.ar; ggarrote@biol.unlp.edu.ar

Keywords: Immunomodulation, Lactobacillus, intestinal epithelial cells, Gastric epithelial cells, Fermented milks

Milk constitutes an excellent carrier for probiotics wherein specific bacterial-fermentation processes may lead to functional products with new beneficial health effects beyond the normal nutritional function of the dairy product itself. These health benefits associated to fermented milk consumption may be originated by the presence of the microorganisms themselves or other bioactive components produced during fermentation such as organic acids, peptides and/or exopolysaccharides (EPS). It has been well documented that certain fermented milks with lactic acid bacteria such as kefir, present the capacity to modulate the host's immune response resulting in an interesting alternative to alleviates symptoms of gastrointestinal inflammatory diseases (1). Considering this, the aim of this work was to evaluate the bioactive properties of the nonbacterial fraction of Lactobacillus paracasei fermented milks with a focus on their ability to modulate the innate immune response on gastric and intestinal epithelial cells growing in culture.

L. paracasei CIDCA 8339 and CIDCA 83124, isolated from kefir grains were characterized as EPS-producing (2) and potentially probiotics strains. These strains are able to resist the gastrointestinal conditions (3, 4), adhere to intestinal epithelial cells (3), downregulate the innate immune response on intestinal epithelial cells (4) and exert a protective effect against Salmonella infection (5). Moreover, it was evidenced that polysaccharide production is influenced by growth temperature without affecting probiotic properties being this aspect of relevance for industrial application (4).

Lactobacilli were inoculated in UHT low fat milk at 5% v/v and incubated at 30°C for 24h. The nonbacterial fractions (NBF) of fermented milks were obtained by centrifugation (5min at 5000g); the supernatants were then neutralized with 5N NaOH and filtered through a 0.45 µm of pore diameter. The lactic and acetic acid concentration in the NBF were determined by HPLC with an AMINEX HPX-87H ion exchange column. To evaluate the immunomodulatory properties of the NBF of fermented products, gastric-epithelial-carcinoma cells (AGS) and reporter intestinal epithelial cells Caco-2-ccl20:luc were treated for 30 min with the NBF and then exposed to stimulation by 1µg/ml Flagellin (FliC) during 8h at 37 °C in an atmosphere of 5% CO2–95% air. Artificially acidified milk (AAM) with lactic and acetic acid, as well as aqueous solutions of lactic acid, acetic acid and EPS isolated from fermented milks were also tested in these systems. A basal condition without any treatment was included as a control; and a FliC stimulated condition was used to define the 100% induction of the proinflammatory response. The innate immune response was define by determination of IL-8 levels in AGS supernatants by ELISA (BD Bioscience OptEIATM human IL-8 ELISA Kit USA) and by measuring luciferase activity in Caco-2-ccl20:luc in a Labsystems Luminoskan TL Plus luminometer (Thermo Scientific, USA) using a luciferase assay system (Promega, USA). IL-8 levels and luminescence were normalized to the stimulated control cells and expressed as a percentage of the normalized average ± standard deviation (SD). In order to evaluate the treatment-induced cytotoxicity, the mitochondrial activity of the cells was evaluated by measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co) to a purple formazan at 550 nm.

The organic acids concentration in the NBF of fermented milks with L. paracasei CIDCA 8339 and CIDCA 83124 were similar, with a value near 100 mM for lactic acid and 12 mM for acetic acid. Both NBF were able to significantly modulate the epithelial innate immune response in vitro. The pre-incubation of the cells with the NBF led to a reduction of an 85–90% of luciferase activity in intestinal cells and a 70% modulation of IL-8 expression in gastric cells (Figure 1, $p<0,05$). Supernatant of AAM with lactic (100 mM) and acetic acid (10 mM) showed the same behaviour as the NBF of fermented milks in Caco-2-ccl20:luc cells indicating that the organic acids can be responsible for the immunomodulation (Figure 1, $p<0.05$). However, in AGS cells supernatant of AAM showed a modulation of IL-8 expression less pronounced (50%) ($p<0.05$). This suggests that although the organic acids are important bioactive metabolites, there might be other metabolites in the NBF that also contribute to the immunomodulatory effect in gastric cells. Lactate solution (100mM) showed a marked modulation of proinflammatory response with a reduction of 80% and 50% in intestinal and gastric cells respectively (Figure 2, $p<0.05$). On the other hand, acetate solution (10mM) induce a reduction of only the 20% of luciferase activity in Caco-2-ccl20:luc cells and no significant modulatory effect was observed in gastric cells. It is important to point out that no significant reduction at the mitochondrial activity was observed

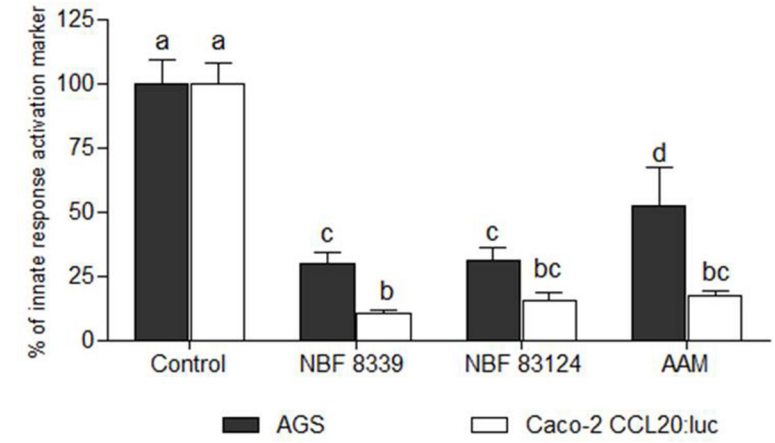


FIGURE 1

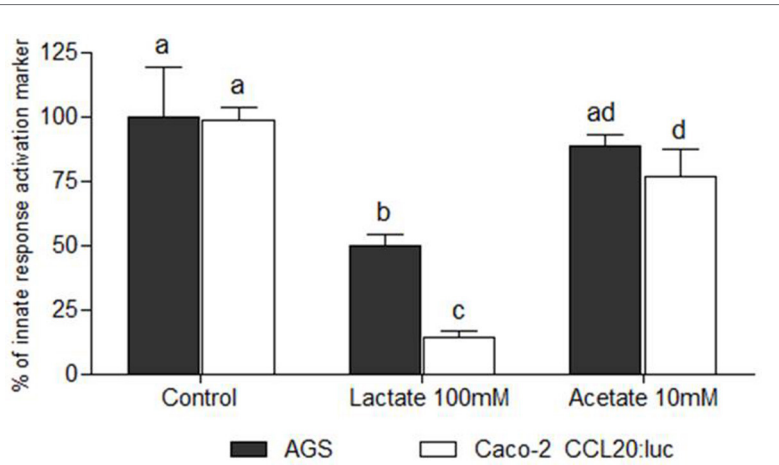


FIGURE 2

on gastric or intestinal epithelial cells preincubated with lactate and acetate solutions. Finally, the effect of EPS solutions on gastric and epithelial cells was evaluated. It has been claimed that EPS can present immunostimulatory or immunosupressive effects

depending on their structure/molecular weight. We have previously evidenced that the EPS synthetized by L. paracasei CIDCA 8339 and CIDCA present different structure (data not shown). Despite this, EPS 8339 and 83124 solutions (200mg/L and 800mg/L) showed similar behaviour on epithelial cells presenting no anti-inflammatory activity even at concentrations much higher than the present in the NBF.

In the present work it was demonstrated that the NBF of fermented milks obtained with L. paracasei CIDCA 8339 and L. paracasei CIDCA 83124 are able to downregulate the innate immune response on gastric and intestinal epithelial cells in vitro and that among the metabolites produce during fermentation, lactate is the main responsible of the immunomodulatory effect. The present results suggest that these fermented milks should be studied in more depth since they could be good candidates to be used as an alternative or complementary treatment for the prevention or relief of symptoms in people that suffer gastritis or inflammatory bowel disease. Regarding this, in vivo models are being carried out for a better characterization of the anti-inflammatory potential of these fermented milks as well as elucidating the main metabolites responsible for the evidenced immunomodulatory capacity.

REFERENCES

1- Iraporda C., Romanin D.E., Rumbo M., Garrote, G.L., Abraham A.G., 2014. The role of lactate on the immunomodulatory properties of the nonbacterial fraction of kefir. Food Research International. 62: 247-253

2- Hamet, M.F., Piermaria, J.A., Abraham, A.G., 2015. Selection of EPS-producing Lactobacillus strains isolated from kefir grains and rheological characterization of the fermented milks. LWT-Food Sci. Technol. 63, 129-135

3- Bengoa, A. A., Zavala, L., Carasi, P., Trejo, S. A., Bronsoms, S., Serradell, M., A., Garrote, G. L., Abraham, A. G., 2017. Simulated gastrointestinal conditions increase adhesion ability of Lactobacillus paracasei strains isolated from kefir to Caco-2 cells and mucin. Food Research International. DOI10.1016/j.foodres.2017.09.093

4- Bengoa, A. A., Llamas, M. G., Iraporda, C., Dueñas, M. T., Abraham, A. G., Garrote, G. L., 2018. Impact of growth temperature on exopolysaccharide production and probiotic properties of Lactobacillus paracasei strains isolated from kefir grains. Food microbiology, 69, 212-218

5- Zavala, L., Golowczyc, M., van Hoorde, K., Medrano, M., Huys, G., Vandamme, P., Abraham, A., 2016. Selected Lactobacillus strains isolated from sugary and milk kefir reduce Salmonella infection of epithelial cells in vitro. Beneicial Microbs, 7, 585-595

MIF and male gender have a fundamental role in innate immunity against Leishmania mexicana infection in a murine model

Alexia Sánchez-Arteaga, Imelda Juárez-Avelar, Tonathiu Rodriguez, Danae Mendoza-Sánchez, Miriam Rodriguez-Sosa

Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico dalexia.02@hotmail.com; imelda_juarez@yahoo.com; tonathiurh@hotmail.com; mendoza.sa.danae@gmail.com; rodriguezm@unam.mx

Keywords: Leishmania mexicana, Pro-inflammatory cytokines, NO2, Balb/c mice, Influence of sex

Background: Macrophage Migration Inhibitory Factor (MIF) is a pleiotropic pro-inflammatory cytokine that works as a sex-linked hormonal immune-modulator. In addition, it is an important molecule in the activation of the immune response against intracellular parasites as Toxoplasma gondii and Leishmania major (L. major), in which the inflammatory immune response-Th1 suppress the parasite growth. This is not the case in Leishmania mexicana (L. mexicana) infection, where it has not been possible to establish an association among the inflammatory immune response-Th1, and the resistance to infection. Furthermore, MIF function and influence of host gender in Leishmania mexicana (L. mexicana) infection is not described.

Objective: To establish the participation of MIF in the innate immune response to L. mexicana infection in a murine model.

Material and Methods: Six- to eight week old BALB/c male (♂) and female (♀) mice genetically deficient of MIF (MIF-/-) and wild-type (MIF+/+), were infected with 2x10e6 promastigotes of L. mexicana subcutaneously in the tail base. 24 and 48 hs post-infection mice were euthanized in excess atmosphere by CO2/O2; subsequently, blood was obtained by cardiac puncture. TNF-α, IL-12, IFN-γ, IL-4, IL-10 and NO2 were measured in serum (systemic response), and supernatants of popliteal ganglia cell (PGC) cultures (local immune response).

Results: In systemic response (serum), MIF-/-♂ and ♀ mice infected with L. mexicana displayed a decreased production of TNF-α and NO2 compared with MIF+/+ ♂ and ♀. However, in supernatants of PGC cultures, MIF-/-♂ and ♀ produced lower

IL-12, IFN-γ and NO2 levels, but higher IL-4 and IL-10 production compared with MIF+/+♂and ♀ 24 h post-infection. In addition, is important to denote that ♂ mice presented significantly higher pro-inflammatory cytokines levels.

Conclusions: These findings suggest the importance of MIF modulation of the initial immune response against L. mexicana infection, due to promoting the early production of pro-inflammatory cytokines (IL-12 and IFN-γ) and NO2, also, it is more efficient in male mice. Is necessary to establish whether the inflammatory-Th1 profile induced early by MIF, support host protection in Leishmania mexicana infection.

Influence of microenvironment of adipose tissue from lean, overweight and obese subjects in the inflammatory phenotype of macrophages

Angelica Girón-Ulloa[1], Erika González-Domínguez[1], Eduardo Patiño-Martinez[1], Hector Adrian Duque-Martinez[1], Salvador Francisco Campos-Campos[2], Roberto González-Amaro[3], Carmen Sánchez-Torres[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico

[2]Hospital Angeles Lindavista, Mexico, Mexico

[3]Facultad de Medicina, Universidad Autónoma de San Luis Potosi, San Luis Potosí, Mexico

gie.ulloa@gmail.com; erikagd1@hotmail.com; epatinom@cinvestav.mx; haduque@cinvestav.mx; drfcampos@prodigy.net.mx; rgonzale@uaslp.mx; csanchez@cinvestav.mx

Keywords: Obesity, Overweight, TNF, Body Mass Index, IL10, M1 macrophages, M2 macrophages, proinflammatory cytokines, SAT, lean, CD163, adipose tissue macrophages, VAT, Tissue remodeling, ITGAX

Introduction: Obesity is a chronic disease characterized by adipose tissue (AT) expansion, accumulation of immune cells and increased production of inflammatory molecules like TNF, IL-6, IL-8, iNOS, CCL2, or ICAM-1. The more representative leukocytes in the AT are the macrophages, and they are a prominent source of pro-inflammatory cytokines such as TNF and IL-6. Macrophages are a heterogeneous cellular population of the innate immunity with different functions and activation profiles. Macrophages can undergo genetic reprogramming in response to environmental cues that drive their differentiation towards a variety of functional phenotypes, where M1 (classical) and M2 (alternative) macrophages represent the endpoints of the activation spectrum. M1 polarization is generated by pro-inflammatory mediators such as IFN-γ and LPS; these macrophages secrete high amounts of proinflammatory cytokines and generate reactive oxygen and nitrogen intermediates. M2 polarization is induced by exposure to Th2 cytokines (IL-4, IL-13) or anti-inflammatory stimuli (IL-10); these macrophages produce high levels of anti-inflammatory cytokines such as IL-10 and IL-1RA, and they are involved in angiogenesis and tissue repair. Adipose tissue macrophages (ATMs) infiltrate the AT during obesity and contribute to inflammation and insulin resistance in mice and humans (1). This accumulation is more notable in visceral adipose tissue (VAT) than in subcutaneous (SAT). Lumeng et al. (2) found a population of ATMs F4/80+CD11c+ in obese mice that were not present in the lean animals. They expressed pro-inflammatory genes and derive from the recruitment of blood monocytes. The authors postulated that ATMs "switch" their phenotype from M2 resident

cells to M1 macrophages during high-fat diet (HFD) induced-obesity. Others authors have reported that ATMs in adipose tissue from obese mice presented a mixed M1/M2 phenotype and remodeling profiles, and that these ATMs become more M2-like with extended HFD-feeding (3). In humans, the characterization of ATM phenotype has been more difficult and the results are contradictory. There are coincident reports about the M2 to M1 "phenotype switch" during obesity (3); others have reported that ATMs in lean subjects have a M1 phenotype that change to M2 during obesity (4). Additionally, there are findings mentioning that ATMs from obese subjects express surface markers of M2 macrophages but they are also capable of secrete pro-inflammatory cytokines (5). Finally, others authors have proposed that ATMs have enhanced capacities of tissue remodeling and angiogenesis in obesity (they express LYVE-1, MMP-9 and PAI-1), whereas the ATMs in lean subjects present a mixed phenotype (6,7). Given the conflicting data on ATM phenotype in lean and obese individuals, in the present study we sought to characterize the phenotype of this cell type in SAT and VAT of healthy lean, overweight and obese donors.

Methods: Samples of SAT and VAT were obtained from lean, overweight and obese patients undergoing laparoscopic appendicectomy or cholecystectomy surgery. Inclusion criteria were: subjects older than 18 years of age, both genders, and metabolically healthy. Some of the tissues were included in O.C.T. to evaluate the monocyte/macrophage markers CD14, CD68, CD163 and CD163L1, and the cytokines TNF, IFN-γ and IL-10 by multicolor Confocal microscopy. Other samples were snap frozen in liquid nitrogen and weighted. RNA extraction from frozen samples was performed, and total RNA was reverse transcribed. Gene expression was evaluated by quantitative real time PCR using pre-design probes and oligonucleotides for CD68, ITGAM, ITGAX, TNF, CD163, CD163L1, IL10, IFNG, IL4, CCL2 and CCR2. Gene expression was expressed relative to TBP and ACTB using the ΔCt method.

Results: The expression of the pan-macrophage markers CD68 and ITGAM (encoding the integrin αM chain, CD11B) was higher in VAT than in SAT of lean subjects, according with the already reported higher abundance of ATMs in VAT. We also found higher expression of pro-inflammatory cytokines, such as TNF and IFN-γ, and lower expression of the anti-inflammatory cytokine IL10 in VAT, which suggest a more prevalent pro-inflammatory state in VAT than in SAT in homeostatic conditions.

CD68 and ITGAM expression was higher in SAT from obese with respect to lean subjects, and their expression correlated with the body mass index (BMI) of the donors. We also observed a higher expression of CCR2 in obese subjects. This fact might be related with the de novo recruitment of circulating monocytes to AT during obesity,

since we detected high density of CD14+ cells in SAT from obese compared with lean individuals. However, no differences were observed in VAT.

The expression of the pro-inflammatory gene ITGAX (encoding the integrin αX subunit, CD11C) was increased in the SAT from overweight and obese individuals compared with lean subjects. The expression of this gene correlated with BMI in lean and overweight donors. Similarly, expression of TNF and IFNG tended to increase in overweight donors and, surprisingly, they correlate negatively with BMI in obesity. This trend was also observed in VAT. Overweight or obese donors did not modify their expression of IL10 mRNA with respect to lean individuals in SAT, but in contrast we detected an augment of IL10 transcripts in VAT that positively correlates with BMI. Finally, we observed that the expression of the M2 markers CD163 and CD163L1 were enhanced in the obese SAT.

Conclusions: There were more significant differences in SAT than in VAT with respect to the numbers and phenotype of ATMs in relation to the degree of adiposity. In overweight subjects we detected the highest levels of pro-inflammatory markers, which could be related with the initiation of an anomalistic expansion of the AT. Our data suggest that obesity is associated with abundance of ATMs only in SAT. These cells seemed to be derived, at least in part, from blood monocytes, presented an anti-inflammatory phenotype and they could be associated with tissue remodeling.

REFERENCES

1. Weisberg SP, et al. 2003. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 112(12), 1796.
2. Lumeng CN, et al. 2007. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J. Clin. Invest. 117(1), 175.
3. Shaul ME, et al. 2010. Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet--induced obesity in mice. Diabetes. 59(5):1171-81.
4. Wentworth JM, et al. 2010. Pro-inflammatory CD11c+ CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. Diabetes 59(7), 1648.
5. Fjeldborg K, et al. 2014. Human adipose tissue macrophages are enhanced but changed to an anti-inflammatory profile in obesity. J. Immunol. Res. 2014, 309548.
6. Aron-Wisnewsky J, et al. 2009. Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J. Clin. Endocrinol. Metab. 94(11), 4619.
7. Bourlier V, et al. 2008. Remodeling phenotype of human subcutaneous adipose tissue macrophages. Circulation 117(6), 806.

Analysis of the inflammasome activation in early and late stages of infection with Dengue virus in a model of endothelial cells

Giovani Visoso Carvajal[1], Gaurav Shrivastava[1,2], Julio García Cordero[1], Leticia Cedillo-Barron[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
[2]University of Alberta, Edmonton, AB, Canada
carvajalgv@gmail.com; shrivast@ualberta.ca; leebeydengue@yahoo.com.mx; lcedillo@cinvestav.mx

Keywords: Dengue Virus, Endothelial Cells, Flavivirus, NLRP3 inflammasome, inflammasome activation

Background: Dengue virus (DENV) is a member of the Flaviviridae family, genus Flavivirus and it causes the disease known as dengue. It possesses a single stranded RNA (ssRNA) genome which is approximately 11 kb in length (1). DENV has a single open reading frame that encodes three structural proteins and seven non-structural proteins. DENV is present in 4 serotypes, DENV 1-4 (2). Dengue virus is able to manipulate and control different processes for its own benefit, such as cell cycle, vesicular traffic, metabolism and mechanisms related to the immune system, like the inflammasome (3). The inflammasome is a group of cytosolic sensors which are expressed by different cells to detect damage or viral infection. The inflammasomes are multiprotein complex involved on immune response (4). Among them, the NLRP3 is the most frequently involved in viral infections, which is constituted by a NOD receptor, the adapter protein (ASC) and procaspase-1. It plays an important role in the innate immune pathway that regulates the processing and secretion of IL-1β and IL-18, both pro-inflammatory cytokines (5). Thereby the activation of the inflammasome serves a central role in the inflammatory response as well as in diverse human diseases. Dengue virus has shown to have the ability to infect different cells, including immune cells (5). Furthermore, it has been reported that many flaviviruses including DENV-2 are able to activate the inflammasome. There are many reports of the participation of DENV in endothelial dysfunction (6). Because alterations in the endothelium have a great impact on severe forms of dengue disease, endothelial cells are an important target of study in the infection by DENV.

Aims: To characterize the expression of the NLRP3 inflammasome components and analyze the activation of the complex in a kinetics with the DENV-2 from early time to late post-infection in a model of HMEC-1 cells.

Materials and Methods: HMEC-1 (A human microvascular endothelial cell line-1) cells were cultured in MCDB131 medium, supplemented with 10% FBS, 1 μg/mL hydrocortisone, 10 ng/mL EGF, 100 U penicillin and L-glutamine, with 100 mg/mL streptomycin at 37°C under 5% CO2. Cells were infected with DENV-2 at a multiplicity of infection (MOI) of 5 at 37 °C for 2 h. The cells were then washed twice with PBS and incubated at 37 °C with fresh growth medium. In this model, HMEC-1 cells grown in a monolayer (6×105 cells/mL) were infected at 3, 6, 12, 24 and 36 hours post infection (hpi) respectively. As control, uninfected HMEC-1 cells were used and cells with heat-inactivated virus (MOCK). Subsequently, the cells were lysed in RIPA buffer for protein extraction and 30 μg of protein was subjected to SDS-PAGE and Western Blot (WB).

The infection was confirmed through WB with the presence of non-structural protein NS5 (RNA polymerase) and the productive infection was evaluated through the plaque assay to extracellular viral titer obtained from the supernatants. Further, the expression of the inflammasome components was analyzed using markers for components of the complex, such as NLRP3 through WB. The activation of the inflammasome was analyzed through the expression of active caspase-1 and caspase-1–dependent IL-1β secretion.

Result: The analysis of the activation of the inflammasome in a model of HMEC-1 cells infected with DENV-2 showed a similar behavior between the expression of its components, its active forms and effector molecules. The expression of inflammasome components such as NLRP3, showed a higher expression in the late stages of infection. We observed a considerable increase in its expression at 24 and 36 hpi, in contrast to mock infected cells, while at 3, 6 and 12 hpi, considered as early stages of infection, we could not observe expression of this protein. This data indicates that DENV-2 promotes an increase in the expression of inflammasome components, which in turn would favor the assembly of the complex and be reflected in the presence of active caspase-1 and IL-1β, both activation marks.

To evaluate the expression of active caspase-1, WB was performed, further to the secretion of IL-1β was quantified through an ELISA assay. The analysis of both proteins showed a similar patron. We found higher expression of active caspase-1 in late post-infection stages (24 and 36 hpi), and a lower expression of this protein in the early stages.

On the other hand, the secretion of IL-1β showed a progressive increase according to the time of infection with DENV-2, showing a maximum peak of secretion in the late stages of infection, this same pattern was observed when analyzing active caspase and the expression of NLRP3. All these components are necessary for the assembly of the complex and its activation, which also shows more activity in the late stages of infection in our model of HMEC-1 cells infected with DENV-2.

Conclussions: All these results suggest that in a model of HMEC-1 cells DENV-2 is able of increase the expression of components of inflammasome and thereby promote higher activation of this complex in later times of infection, however, little is known yet about what could be happening, some reports suggest that it could participate in the pathogenesis of the disease or favor viral replication.

REFERENCES

(1). Screaton et al., 2015. New insights into the immunopathology and control of dengue virus infection. Nature Reviews Immunology 15, 745-759.

(2). Diamond et al., 2015. Molecular Insight into Dengue Virus Pathogenesis and Its Implications for Disease Control. Cell 162. Vol. 162, Issue 3. p488–492.

(3). Fischl W, Bartenschlager R. 2011. Exploitation of cellular pathways by Dengue virus. Curr Opin Microbiol. Aug;14(4):470-5.

(4). Jo et al., 2015. Molecular mechanisms regulating NLRP3 inflammasome activation. Cellular & Molecular Immunology 13, 148–159.

(5). Wu et al., 2013. CLEC5A is critical for dengue virus-induced inflammasome activation in human macrophages. Blood;121(1):95-106.

(6). Dalrymple and Mackow. 2012. Endothelial Cells Elicit Immune-Enhancing responses to Dengue Virus Infection. J Virol. 86(12):6408-1.

Lactobacillus fermentum UCO-979C beneficially modulates the innate immune response triggered by Helicobacter pylori infection

Valeria García-Castillo[1,2,3], Hortensia Zelaya[2], Alejandra Ilabaca[3], Marcela Espinoza-Monje[3], Ryoya Komatsu[1], Leonardo Albarracin[1,2], Haruki Kitazawa[1,4], Apolinaria García[3], Julio Villena[1,2]

[1]Tohoku University, Aoba-ku, Sendai, Japan
[2]Centro de Referencia para Lactobacilos, San Miguel de Tucuman, Argentina
[3]University of Concepción, Concepción, Chile
[4]International Education and Research Center for Food and Agricultural Immunology (CFAI), Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
valeriagarcia@udec.cl; hzelaya@cerela.org.ar; aleilabaca@udec.cl; marespinozamonje@gmail.com; ryoya.komatsu.s5@dc.tohoku.ac.jp; lalbarracin@facet.unt.edu.ar; haruki.kitazawa.c7@tohoku.ac.jp; apgarcia@udec.cl; jcvillena@cerela.org.ar

Keywords: Helicobacter pylori, Inflammation, cytokine profile, immunobiotics, Lactobacillus fermentum UCO-979C

Helicobacter pylori infection is a major cause of chronic gastritis and peptic ulcer and a risk factor for gastric malignancies. Although antibiotics-based eradication is effective, this treatment is expensive and causes side effects and antibiotic resistance. Probiotics could present a low-cost, large-scale alternative solution to prevent or decrease the colonization of this gastric pathogen. In fact, some clinical trials have demonstrated that probiotics are effective as adjunct treatment in H. pylori infections and prophylaxis, although deeper studies are necessary to fully understand the mechanisms of action involved in their beneficial effects. In this regard, we have performed a detailed characterization of the probiotic properties and the anti-H. pylori activity of Lactobacillus fermentum UCO-979C isolated from human stomach. We demonstrated that the UCO-979C strain is able to tolerate acid and bile salts; produces hydrogen peroxide; and has high hydrophobicity properties. In addition, we observed that it is able to efficiently adhere to gastric mucosa as demonstrated by in vitro (gastric adenocarcinoma human (AGS) cells) and in vivo (Mongolian Gerbils) experiments. Moreover, we found that L. fermentum UCO-979C strongly inhibited the adhesion, growth and urease activity of H. pylori (Garcia-Cancino et al, 2017 Electronic J Biotechnol 25:75–83). Preliminary studies in AGS cells also showed that L. fermentum UCO-979C significantly reduced H. pylori-induced IL-8 production. However, no deeper immunological studies were performed with this strain. Therefore, the aim of this work was to analyze whether L. fermentum UCO-979C beneficially modulates the innate immune response triggered by H. pylori infection in human gastric epithelial cells and macrophages.

The capacity of L. fermentum UCO-979C to modulate immune responses in AGS cells and human THP-1 macrophages was evaluated. For this purpose, AGS cells or PMA-differentiated THP-1 macrophages were treated with different doses of L. fermentum UCO-979C (105, 106 or 107 cells/ml) and then challenged with H. pylori ATCC43504 (107 cells/ml). Cellular damage and levels of TNF-α, IL-1β, IL-6, TGF-β, CCL20 and IL-8 were determined 24 hours after the challenge in AGS cells. Cellular damage and levels of TNF-α, IL-1β, IL-6, IL-10, IL-12p70, IL-12p40 and IFN-γ were determined 24 hours after the challenge in THP-1 macrophages.

It was observed that L. fermentum UCO-979C is able to protect AGS cells against H. pylori-induced inflammatory damage in a dose dependent manner. A higher viability (MTT-%: UCO979C=95, control=73) and a decreased cytotoxicity (LDH-UI/L: UCO979C=56.4±3.7, control=125.7±4.6) was observed in lactobacilli-treated AGS cells infected with H. pylori when compared to the infected control. In addition, a significant decrease in the production of pro-inflammatory cytokines and chemokines (IL-1β, IL-6, CCL20 and IL-8) and an increase in the immunoregulatory cytokine TGF-β was observed in UCO-979C-treated cells when compared to the infected control. The most notorious effects were achieved by the smaller dose of L. fermentum UCO-979C (TNF-α-pg/mL: UCO979C=73.2±3.6, control=195.8±3.6; TGF-β-pg/mL: UCO979C=195.7±5.9, control=255.9±5.5). L. fermentum UCO-979C was also able to differentially modulate the inflammatory response triggered by H. pylori in THP1 macrophages. A decreased cytotoxicity (LDH-UI/L: UCO979C=167.3±2.5, control=235.9±3.6) and a higher viability (MTT-%: UCO979C=93, control=84) were detected in UCO-979C-tretaed THP1 macrophages after the challenge with the pathogen when compared to the infected control. The inflammatory cytokines TNF-α and IL-1β were significantly lower in lactobacilli-treated macrophages than in controls after H. pylori challenge (TNF-α-pg/mL: UCO979C=316.5±3.2, control=456.7±3.3). On the contrary, the Th1 cytokines IL-12p70, IL-12p40 and IFN-γ and the immunoregulatory cytokine IL-10 were significantly higher in UCO-979C-treated THP1 cells than in control cells after H. pylori challenge (IFN-γ-pg/mL: UCO979C=342.9±3.4, control=291.4±2.3; IL-10-pg/mL: UCO979C=456.8±3.1, control=325.7±3.5).

These findings provide evidence of the beneficial effects of L. fermentum UCO-979C against the inflammatory damage induced by H. pylori infection in both epithelial and immune cells. Although our findings should be proven in appropriate experiments in vivo, in both H. pylori infection animal models and human trials, the results of the present work provide a scientific rationale for the use of L. fermentum UCO-979C to prevent or reduce H. pylori-induced gastric inflammation in humans.

Comparative study of the immunomodulatory activities of lactobacilli strains in porcine intestinal epithelial cells: effect on the innate antiviral immune response

Leonardo Albarracin[1,2], Valeria García- Castillo[1,2,3], Hikaru Iida[1], Nana Sato[1], Paulraj Kanmani[1,4], Alejandra Ilabaca[3], Hisashi Aso[1,4], Apolinaria García[3], Haruki Kitazawa[1,4], Julio Villena[1,2]

[1]Tohoku University, Aoba-ku, Sendai, Japan
[2]Centro de Referencia para Lactobacilos, San Miguel de Tucuman, Argentina
[3]University of Concepción, Concepción, Chile
[4]International Education and Research Center for Food and Agricultural Immunology (CFAI), Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
lalbarracin@facet.unt.edu.ar; valeriagarcia@udec.cl; hikaru.iida.s7@dc.tohoku.ac.jp; nana.sato.s2@dc.tohoku.ac.jp; paulrajkanmani@gmail.com; aleilabaca@udec.cl; asosan@tohoku.ac.jp; apgarcia@udec.cl; haruki.kitazawa.c7@tohoku.ac.jp; jcvillena@cerela.org.ar

Keywords: TLR3, biomarkers, antiviral response, immunobiotics, PIE cells

Lactobacillus rhamnosus CRL1505 and L. plantarum CRL1506 are immunomodulatory probiotic strains (immunobiotics) with the ability to improve the intestinal antiviral response and the protection against viral infections as demonstrated in animal models and clinical trials. In order to advance in the understanding of the mechanisms involved in the antiviral capacities of both immunobiotic strains, we have previously performed comparative transcriptomic studies in porcine intestinal epithelial (PIE cells). These cells were stimulated with L. rhamnosus CRL1505 or L. plantarum CRL1506 and then challenged with the TLR3 agonist poly(I:C). The immunotranscriptomic response of PIE cells was evaluated 12 h after poly(I:C) challenge. Our results showed that both immunobiotic strains significantly improved the expression of IFN-α and IFN-β as well as the antiviral factors RNASE4, RNASEL, OAS1, OASL, MX1, and MX2 when compared to controls. In addition, both lactobacilli strains increased the expression of cytokines (IL-1β, IL-6), chemokines (CCL4, CCL20, CCL28, AMCF-II, CXCL10), and enzymes involved in prostaglandin biosynthesis (PTGES, PTGER4). For the majority of these genes, their expression in CRL1505-tretaed PIE cells was significantly higher than in cells treated with the CRL1506 strain. Furthermore, only L. rhamnosus CRL1505 differentially regulated the expression of CXCL2, CXCL5, and CXCL11. Then, our transcriptomic analysis successfully allowed us to identify a group of genes that can be used as prospective biomarkers for the screening of new antiviral immunobiotics in PIE cells (Albarracin et al, 2017, Front Immunol 8:57).

The aim of this work was to evaluate whether the immunotranscriptomic changes induced by L. rhamnosus CRL1505 and L. plantarum CRL1506 in PIE cells are unique and not sheared by non-immunomodulatory strains of the same species. For this purpose, we evaluated the effect of different L. rhamnosus and L. plantarum strains on the antiviral response of PIE cells. The immunomodulatory L. rhamnosus IBL027, and L. plantarum MPL16 and the non-immunomodulatory L. rhamnosus CRL489, L. rhamnosus CRL576, and L. plantarum CRL681 strains were used. The CRL1505 and CRL1506 strains were used as positive controls. PIE cells were seeded at 104 cells per well in 12-well type I collagen-coated plates and stimulated with lactobacilli (108 cells/ml) for 48 h. Then cells were challenged with poly(I:C) (60 μg/ml) for 12 h. Two-step real-time qPCR was performed to characterize the expression of biomarker genes in PIE cells. The following genes were evaluated: IFN-α, IFN-β, A20, TLR3, RIG-I, RNASEL, MX2, OAS1, CCL4, CXCL5, IL-15, EPCAM, SELE, SELL, PTGS2, PTGER4, PLA2G4A, and PTGES.

L. plantarum MPL16, a strain that is able to modulate bacterial-mediated inflammation in PIE cells, induced an immunotranscriptomic profile that was similar to the observed for L. rhamnosus CRL1505 with increases of IFN-β, A20, RNASEL, MX2, OAS1, as well as CXCL5, EPCAM, SELE, PTGS2, and PLA2G4A. The expression of those genes in CRL1505- and MPL16-treated PIE cells was significantly higher than in the other groups. Of interest, L. rhamnosus IBL027, a strain that is able to improve the immune response to mucosal antigens, induced an immunotranscriptomic profile in PIE cells that was similar to the observed for L. plantarum CRL1506. The non-immunomodulatory L. rhamnosus CRL489, L. rhamnosus CRL576, and L. plantarum CRL681 strains induced modest increases in IFN-α, IFN-β, SELE, and SELL, while the other studied genes were not different from control PIE cells challenged with poly(I:C).

The results of this work confirm that the effect of L. rhamnosus and L. plantarum strains on the innate antiviral immune response of PIE cells is a strain-specific property. Moreover, our study indicates that the set of biomarkers genes (IFN-α, IFN-β, A20, TLR3, RIG-I, RNASEL, MX2, OAS1, CCL4, CXCL5, IL-15, EPCAM, SELE, SELL, PTGS2, PTGER4, PLA2G4A, and PTGES) would allow an efficient screening of new antiviral immunobiotics in PIE cells. This study is of importance since it verified that these biomarker genes are able to bluntly allow the selection of immunobiotic strains with the ability to beneficially modulate the innate antiviral immune response.

REFERENCES

Albarracin, L., Kobayashi, H., Iida, H., Sato, N., Nochi, T., Aso, H., Salva, S., Alvarez, S., Kitazawa, H., and Villena, J. 2017. Transcriptomic analysis of the innate antiviral immune response in porcine intestinal epithelial cells: Influence of immunobiotic lactobacilli. Front. Immunol. 8(FEB). doi:10.3389/fimmu.2017.00057.

Immunomodulatory properties of microcapsules containing Lactobacillus rhamnosus UCO-25A in planktonic o biofilm forms: impact on intestinal and systemic immunity

Alejandra Ilabaca[1], Patricia Clua[2], Susana Salva[2], Valeria García[1,2,3], Leonardo Albarracin[2], Haruki Kitasawa[3], Susana Alvarez[2], Apolinaria Garcia-Cancino[1], Julio Villena[2,3]

[1]University of Concepción, Concepción, Chile
[2]Centro de Referencia para Lactobacilos, San Miguel de Tucuman, Argentina
[3]Tohoku University, Aoba-ku, Sendai, Japan
alejandrailabacaf@gmail.com; aleilabaca@udec.cl; ssalva@cerela.org.ar; valeriagarcia@udec.cl; albarracin@cerela.org.a; haruki.kitasawa.c7@tohoku.ac.jp; salvarez@cerela.org.ar; apgarcia@udec.cl; jcvillena@cerela.org.ar

Keywords: Probiotics, Biofilm, encapsulation, inmune system, inmubiotics

Previously, we performed a detailed characterization of the probiotic properties of Lactobacillus rhamnosus UCO-25A originally isolated from human stomach. We demonstrated that the UCO-25 strain has high hydrophobicity properties and is able to form biofilms on glass and cell surfaces. Moreover, we demonstrated that L. rhamnosus UCO-25A is a strain with immunomodulatory activity. Using a murine model, we observed that the oral administration of UCO-25A strain is able to increase the activity of peritoneal macrophages and intestinal antigen presenting cells as well as the levels of intestinal IgA (Ilabaca et al., 2018, submitted for publication). Then, our findings provided a scientific rationale for the use of L. rhamnosus UCO-25A in the development of new immunobiotic products. One of the major challenges in the production of new food or pharmaceutical products is to preserve the viability of immunobiotic strains in order they can exert their beneficial effects. Encapsulation is an interesting alternative to achieve this goal. Encapsulation can protect microorganisms from external environments and induce the release of them in a metabolically active state in the intestine.

The aim of this work was to evaluate whether L. rhamnosus UCO-25A, administered in an encapsulated form, does preserve its immunomodulatory activities. Moreover, the immunomodulatory capacities of its planktonic o biofilm states were comparatively studied. Immunobiotic capsules were prepared with carob tree-gum and alginate through an extrusion process in the B-390/B395Pro Encapsulator. Capsules were then covered with low molecular weight chitosan (Type I). Female 5-week-old BALB/c mice were used for in vivo studies. L. rhamnosus UCO-25A

encapsulated in planktonic (P25A) or biofilm (B25A) forms were administered at a concentration of 109 cells/mouse/day to different groups of mice for two consecutive days by gavage. Mice treated with PBS solution were used as controls. One day after the last treatment we evaluated: a) peritoneal macrophages phagocytic and microbicidal activities; b) IgA levels in intestinal fluid (IF); c) levels of IFN-γ and TNF-α in IF and serum, and d) immune cell populations in peritoneal cavity, Peyer´s patches and spleen by flow cytometry. Although P25A or B25A did not significantly change the number of peritoneal macrophages (F4/80+ cells) both treatments improved their phagocytic and microbicidal activity (% of phagocytosis: control=4.2±1.2, P25A=19.8±2.4, B25A=16.8±2.1) as well as their MHC-II expression. In addition, enhanced levels of IF and serum TNF-α and IFN-γ were detected, indicating a potent systemic stimulation as well (serum IFN-γ (pg/mL): control=65.2±4.3, P25A=187.3±4.7, B25A=145.6±3.9). It should be noted that P25A was more efficient to increase those parameters when compared to B25A. We also observed changes in antigen presenting cells of mice Peyer´s patches. Higher percentages of CD11b+CD86+ as well as CD11c+CD86+ cells were detected in mice treated with P25A or B25A when compared to controls. Both treatments were equally effective to modulate antigen presenting cells (% of CD86+ in CD11c+ cells: control=72.4±3.7, P25A=88.1±4.1, B25A=85.6±3.3). P25A or B25A were also able to stimulate intestinal humoral immunity as observed by the improved levels of IF IgA (IgA (ug/mL): control=2.1±0.2, P25A=3.1±0.1, B25A=2.7±0.1) and the percentages of B220+ and CD24+ cells in Peyer´s patches (% of B220+ cells: control=18.3±2.3, P25A=32.5±3.1, B25A=23.4±2.7). Once again, the P25A treatment was more efficient to increase those parameters when compared to B25A. No significant influence of P25A or B25A was observed on T cells as demonstrated by studies of CD4+ and CD8+ cells in Peyer´s patches and spleen.

The results of this work show that the UCO-25A strain, encapsulated in a planktonic state, has optimal immunomodulatory properties demonstrated by its ability to stimulate immune cells in both intestinal and systemic compartments. These findings open the possibility to develop prototype immunobiotic products using encapsulated planktonic L. rhamnosus UCO-25A intended for use in humans.

Lower HDL levels during HIV-1 infection are associated with increased inflammatory markers and disease progression

Damariz Marin-Palma[1,2], Gustavo A. Castro[2], Jaiberth A. Cardona[1,2], Silvio Urcuqui-Inchima[2], Juan C. Hernandez[1]

[1]*Universidad Cooperativa de Colombia, Bogotá, Colombia*
[2]*Universidad de Antioquía, Medellín, Colombia*
bleidy1122@gmail.com; gustavoandresc@gmail.com; jaiberth.cardona@campusucc.edu.co; silvio.urcuqui@udea.edu.co; juankhernandez@gmail.com

Keywords: C-Reactive Protein, HIV-1, Inflammasomes, Inflammation, NLRP3, High-density lipoproteins, AIDS

Introduction: High-density lipoproteins (HDL) are responsible for the efflux and transport of excessive cholesterol from peripheral tissues to the liver (1). In addition, HDL can modulate various immunological mechanisms, including the inflammatory response, apoptosis and oxidative stress. One of the main mechanisms involve in the development of inflammatory responses is the inflammasome activation. Inflammasomes are multiprotein complexes, that have been reported to be activated in response to several stimuli, and even during HIV-1 infection (2), thus contributing to immune hyperactivation. Among the HIV-1 pathogenesis, chronic and uncontrolled inflammatory response is the main mechanism of AIDS progression (3,4). Previous reports suggest that HDL can modulate the inflamasomes activity in vitro. However, the relationship between HDL and inflammasome in the context of HIV-1 infection is unclear. Therefore, this research aims to explore the association between HDL and the components of inflammatory response during HIV-1 infection.

Methodology: A cross-sectional study, including 36 HIV-1-infected patients without antiretroviral treatment or active coinfections, and 36 healthy controls matched by sex and age, was conducted (Table 1). Viral load, CD4+ T-cell counts, serum HDL and C-reactive protein (CRP) were quantified. Serum cytokine levels, including IL-1β, IL-6 and IL-18, were measured by ELISA. The inflammasome-related genes in peripheral blood mononuclear cells were assessed by quantitative real time PCR. Statistical analysis was done by using Mann-Whitney U, Kruskal-Wallis and Spearman correlation test.

Table 1: Demographic features of enrolled individuals

	HIV-1-infected patients (n=36)	Healthy donors (n=36)
Women : Men	17:19	17:19
Age in years: Median	28	30
CD4+ T-cells/mm^3: Median	629	791
Viral load copies/mL: Median	12,552	N/A

Results: HIV-1-infected patients showed a significant decrease in HDL levels, particularly those subjects with higher viral load and lower CD4+ T-cell counts. No evident changes were observed in other parameters of the lipid profile. Moreover, up-regulation of inflammasome-related genes (NLRP3, AIM2, ASC, IL-1β and IL-18) was observed, notably in those HIV-1-infected individuals with higher viral loads (above 5.000 copies/mL), in comparison to healthy donors. Serum levels of IL-6 and CRP were also elevated in HIV-1-infected individuals, compared to the controls. Significant negative correlations between HDL and the mRNA of NLRP3, AIM2, ASC, IL-1β and IL-18, as well as viral load and CRP were observed in HIV-1-infected subjects. Likewise, a significant positive correlation between HDL and CD4+ T-cell counts was found.

Conclusion: In summary, the results shown that HDL may modulate the expression of several key components of the inflammatory response (including inflammasome components) during HIV-1 infection, suggesting a novel role of HDL in modifying the inflammatory state and consequently, the progression of HIV-1 infection.

REFERENCES

1. Marín-Palma D, Urcuqui-Inchima S, Hernández JC. Inflamación y respuesta inmune innata: Participación de las lipoproteínas de alta densidad. IATREIA. 2017 Oct-Dic;30(4):423-35 10.17533/udea.iatreia.v30n4a06
2. Hernandez JC, Latz E, Urcuqui-Inchima S. HIV-1 induces the first signal to activate the NLRP3 inflammasome in monocyte-derived macrophages. Intervirology. 2014;57(1):36-42 10.1159/000353902
3. Grossman Z, Meier-Schellersheim M, Paul WE, Picker LJ. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nat Med. 2006 Mar;12(3):289-95 10.1038/nm1380.
4. Boasso A, Shearer GM. Chronic innate immune activation as a cause of HIV-1 immunopathogenesis. Clinical immunology. 2008 Mar;126(3):235-42 10.1016/j.clim.2007.08.015.

Elucidating natural variations in the proinflammatory response of the domesticated bovine using an endotoxemia model

Jeffery A. Carroll[1], Nicole C. Burdick Sanchez[1], Paul R. Broadway[1], Thomas H Welsh Jr.[2], Ronald D. Randel[2]

[1]Agricultural Research Service (USDA), Washington D.C., United States
[2]Texas A&M AgriLife Research, Texas A&M University, Dallas, United States
jeff.carroll@ars.usda.gov; nicole.sanchez@ars.usda.gov; rand.broadway@ars.usda.gov; twelsh@cvm.tamu.edu; ron.randel@ag.tamu.edu

Keywords: Endotoxemia, innate immunity, bovine, Natural variations, Proinflammatory response

The innate immune system is an evolutionarily ancient and highly conserved mechanism designed to fight disease within the body. It represents the antigen-nonspecific defense mechanisms of the immune system that are elicited immediately or within several hours (0 to 4 hours) after exposure to an antigen. Beyond the physical barriers such as skin, mucosal secretions, tears, urine, stomach acid and cilia, the innate immune system serves as the first line of defense for providing immunological protection. When functioning properly, the innate immune system eliminates the majority of pathogenic organisms that elude the body's physical barriers and enter into the animal. While the term "innate immunity" implies that this component of the immune system is stable or resolute, this is not always true. In fact, while the innate immune system is always present to some degree, it can be either enhanced or compromised due to numerous factors such as wounds, dehydration, nutritional restriction, genetic predispositions, exposure to stress, and/or natural variations that exist within a population. Through a series of research experiments, we have documented the existence of naturally occurring variations in the proinflammatory response of the innate immune system in cattle following an i.v. challenge with a bolus dosage of endotoxin. Specifically, we have demonstrated differences in the proinflammatory response due to gender and animal flightiness.

In a study that evaluated the proinflammatory response in prepubertal Bos indicus calves, six healthy pure-bred Brahman heifers and five healthy purebred Brahman bulls (average age = 253 ± 19.9 and 288 ± 47.9 days; average body weight = 194 ± 11 kg and 247 ± 19 kg for heifers and bulls, respectively) were challenged i.v. with an Escherichia coli-derived lipopolysaccharide (LPS) at a dose of 0.25 μg/kg body weight at 0 h. Blood samples for serum were collected at 30-min intervals and whole blood samples were collected at 1-h intervals from −2 to 8 h, and at 12 h and 24 h relative to the LPS

challenge. One trained observer assessed and recorded each animal's sickness behavior score by visual observation immediately prior to each blood collection. Calves were also fitted with rectal temperature (RT) monitoring devices that recorded RT at 1-min intervals throughout the duration of the study. Results from this study revealed some significant biological differences in the proinflammatory response between heifers and bulls. Specifically, heifers maintained greater RT ($P<0.01$) and serum concentrations of tumor necrosis factor-alpha (TNF; $P<0.01$) compared to bulls. However, sickness scores ($P<0.01$) and serum concentrations of interferon-gamma (IFN; $P<0.03$) were greater in bulls than in heifers. Serum concentrations of cortisol and interleukin-1 beta (IL-1), were not different ($P>0.10$) between heifers and bulls. Additionally, circulating lymphocytes and neutrophils were not different ($P<0.10$) between heifers and bulls post-LPS challenge. Given that the calves used in this study were prepubertal, we speculate that the differences observed in the proinflammatory response are independent of sex hormones, and most likely reflect conserved sexually dimorphic proinflammatory responses.

To determine the potential impact of animal flightiness (i.e., temperament) on the proinflammatory response of cattle, 24 purebred Brahman yearling bulls (10 mo of age) were selected based on temperament score which is calculated by taking the average of the exit velocity (EV; flight speed) and pen score (PS) for each animal. Bulls were ranked into 3 groups: Calm, lowest temperament score (n=8; 0.87 m/s EV and 1 PS), Intermediate (n=8; 1.59 m/s EV and 2.25 PS), and Temperamental, highest temperament score (n=8; 3.70 m/s EV and 4.88 PS). Bulls were fitted with indwelling jugular catheters and RT devices that recorded RT at 1-min intervals. The following day blood samples were collected at 30-min intervals from -2 to 8 h relative to an i.v. infusion of LPS (0.5 μg/kg body weight) at 0 h. Each animal was assessed by a trained observer and a sickness behavior score was assigned based on visual observation immediately prior to each blood collection. Results from this study demonstrated that the profile of the proinflammatory response to the LPS challenge was significantly dependent upon the animal's temperament classification. Prior to LPS administration, basal RT was greater ($P<0.01$) in the Temperamental bulls compared to the Calm bulls, as was pre-LPS concentration of cortisol. However, the magnitude of the LPS-induced febrile response was greater ($P<0.05$) in Calm bulls compared to Temperamental bulls. Serum concentration of epinephrine was greater ($P<0.01$), both pre- and post-LPS in Temperamental bulls compared to Calm bulls. While serum concentration of TNF was greater ($P< 0.01$), serum concentrations of IL-6 and IL-4 were lesser ($P<0.01$) in Temperamental bulls compared to Calm bulls. Interestingly, sickness scores were greater ($P<0.01$) in the Calm bulls compared to the Temperamental bulls, with the Temperamental bulls exhibiting virtually no visual signs of illness. These data demonstrate divergent proinflammatory responses in cattle that can be ascribed to their temperament classification.

Collectively, these studies clearly demonstrate that naturally occurring variations exist in the proinflammatory response of the bovine. This is particularly interesting given the highly conserved nature of the innate immune system. Elucidating the existence of naturally occurring variations in immune system function within a genetically similar population will aid in developing management strategies and animal health programs that can improve animal health and well-being.

Teaching immunology based on open science´s principles

Jairo Lumpuy-Castillo, Edel Jiménez-Hernández, Cristobal González-Losada, José Alejandro Rodríguez-Pérez, Alberto Juan Dorta-Contreras

Universidad de Ciencias Médicas de la Habana, Havana, Cuba
jlumpuy@gmail.com; jimenez_edel@yahoo.es; chriss.losada@gmail.com; frcala@infomed.sld.cu; adorta@infomed.sld.cu

Keywords: Open Science, Medical Education, Open Data, teaching immunology, medical scholarship

Introduction: Open Science was a recent phenomenon and it takes part of the movement of Open Access for publishing in order to try to democratize science for all. In spite of its detractors, open science offers tools, working flow for make a transparent and reproducible research. This movement proposes that the scientific knowledge could be freely accessible and transferred.(Kittrie et al., 2017; Watson, 2015)

Quincke's Research Scholarship (QRS) is a selective and inclusive summer course that it fundamentals are based on open science´s principle. This course highly novel since its pedagogical point of view is devoted to undergraduate students medical residents and PhD students.(Dorta-Contreras, 2017)

The course was established in memory of Heinrich Quincke, a German scientist, who was the first to study cerebrospinal fluid in 1891. The summer course takes place in Laboratorio Central de Líquido Cefalorraquídeo (LABCEL), it is a science and technological entity that is part of Universidad de Ciencias Medicas de la Habana and located in Facultad Miguel Enríquez. (Dorta-Contreras, 2017; Gonzalez-Argote et al., 2016)

Method: The students and residents were selected over the base of their records and its curriculum vitae mainly supported by some previous research experience like the participations in scientific meetings for medical students or not, papers published and awards.

QRS is a one week summer course, it has two different levels. One of them is devoted to undergraduate medical students and the other one is for residents from all the different medical specialties and PhD students.

The employed modality is based on open science´s principles. On the first day of the course, the students received primary non-published original data from a research done by researchers from LABCEL with the cooperation with the Neurochemistry Lab, Goettingen Georg-August University, Germany and the Immunology Department Aarhus University, Denmark. During the week the students have to develop the working hypothesis from the research data converting the data into scientific understanding.

Open data are from some original research about molecules recently described that takes part of the lectin complement pathway in the brain. This complement cascade is now under discovering. The students have to solve the question marks about the dynamics of blood-brain diffusion, the different aggregation and polymerization structures of the molecule by drawing upon the theoretical conferences done by several specialist researchers and laboratory work they pursue as part of the open science effort. This process was accompanied by LABCEL medical assistant students.

The students work in little teams and they can feel the same sensation of the discovering process simultaneously like the researchers with the advances of this particular science field.

Results and Discussion: Since 2012, ninety-two students have attended the QRS, including Cuban and foreign undergraduate students and medical residents. Table 1 shows the distribution of students by year and countries participants.

Due to the total LABCEL capacity the total of students by each type could not be larger than 15. The medical residents started to participate in the second edition due to their demand. In 2016's version no residents were included in the announcement.

Table 1: Undergraduate medical students and medical residents by year and countries participants

Year	Undergraduate students	Countries	Medical residents	Countries
2012	7	Cuba	-	-
2013	14	Cuba, China	1	Cuba
2014	12	Cuba, Vietnam	8	Cuba, Uruguay, Bolivia
2015	12	Cuba, Mexico	6	Cuba
2016	11	Cuba	-	-
2017	14	Cuba	7	Cuba, Bolivia, USA
Total	70		22	

The request for QRS is increasing by year but the capacity of the lab and the selection method does not allow extending the course for more students. Also in 2016 one student from Harvard was selected but finally did not come. In the Sixth Edition professors from Spain were participated. Two outstanding professors from Navarra University gave lectures.

This kind of course in which the medical students and residents became protagonists of a scientific solution has been very well accepted among the participants. Because of this process the students learn to work in team groups, increase the capacity for solve practical problems by studying and applying the supplied theory and the practice with creativity, learn how to do some immunological techniques to complement the information they need.(Alberto Juan Dorta-Contreras et al., 2017; Garcia-Rivero and González-Argote, 2017; Maseda, 2013)

The students that want to continue working in the lab, a post-Quincke space was created in LABCEL. These students and the assistant medical students can be nominated to the Local Undergraduate Research Award in Physiology (LURAP) done yearly by the American Physiological Society (APS) since 2016.

In 2017 QRS receive the International Opportunity Program (IOP) Award for two years in order that the students can apply for a travel grant to participate. This award was also done by APS.

QRS received the Annual Immunology Award 2015 by the Cuban Society of Immunology and it is now applying for Educación Médica Award supported by Lilly Foundation and Universidad Complutense de Madrid.

Conclusion: QRS is an innovative approach to teaching Immunology for medical students and residents making accessible science and it constitute a good way to appropriate open science's principles.

REFERENCES

Alberto Juan Dorta-Contreras, Padilla-Docal, B., Martínez-Larrarte, J. P., Iglesias-González, I., Cárdenas-de-Baños, L., Castillo-González, W., et al. (2017). Open Science for Medical Students: Quincke's Research Scholarship. FASEB J. 31, 576.56-576.56. Available at: http://www.fasebj.org/content/31/1_Supplement/576.56.abstract?sid=55ce683c-6c3e-4651-861f-a326e6ade54d [Accessed October 19, 2017].

Dorta-Contreras, A. J. (2017). Ciencia abierta para estudiantes de medicina: becas de investigación Quincke. Educación Médica 18, 149. doi:10.1016/j.edumed.2016.09.002.

Garcia-Rivero, A. A., and González-Argote, J. (2017). Formas de hacer ciencia. Educación Médica 18, 209–211. doi:10.1016/j.edumed.2016.03.010.

Gonzalez-Argote, J., Garcia-Rivero, A. A., and Dorta-Contreras, A. J. (2016). El padre de la Neuroinmunología. Neurología Argentina 8, 296–303. doi:10.1016/j.neuarg.2016.06.004.

Kittrie, E., Atienza, A. A., Kiley, R., Carr, D., MacFarlane, A., Pai, V., et al. (2017). Developing international open science collaborations: Funder reflections on the Open Science Prize. PLOS Biology 15, e2002617. doi:10.1371/journal.pbio.2002617.

Maseda, B. (2013). Los Catorce de Dorta. Revista Juventud Técnica. Available at: http://www.cubahora.cu/ciencia-y-tecnologia/los-catorce-de-dorta [Accessed October 5, 2017].

Watson, M. (2015). When will "open science" become simply "science"? Genome Biol 16, 101. doi:10.1186/s13059-015-0669-2.

Phenotypic and functional characterization of macrophages subpopulations CD3+ TCRαβ+/ TCRαβ-

Adriana Rodriguez-Cruz[1], Lucero Ramón-Luing[1], Claudia Carranza[1], Ricardo Lascurain-Ledesma[2], Valerie F.J. Quesniaux[3], Bernhard Ryffel[3], Irene Garcia[4], Leslie Chavez-Galan[1]

[1]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
[2]Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[3]University of Orléans, Orléans, France
[4]Université de Genève, Geneva, Switzerland
adrianardzcruz@gmail.com; ramonluing@yahoo.com.mx; carranza.salazar.claudia@gmail.com; rlascurainl@yahoo.com.mx; quesniaux@cnrs-orleans.fr; bryffel@cnrs-orleans.fr; Irene.Garcia-Gabay@unige.ch; lchavez_galan@iner.gob.mx

Keywords: Tuberculosis, TNF, BCG Bacillus Calmette Guerin, CD3+TCRαβ+ macrophage, CD3+TCRαβ+ monocyte, TCR in myeloid cells

Background: TCR expression is considered exclusive of lymphoid origin. Nevertheless, this variable receptor has also been found in cells of myeloid origin (1,2,3). Using monocyte derived macrophages (MDM) from healthy donors, and BCG-infected, it was observed that the percentage of TCR+ macrophages increased in response to the infection and apparently this subpopulation is TNF-dependent (1). Preliminary results from our group showed that after 2 weeks of BCG-infection there is an increase in the recruitment of two myeloid subpopulations: CD11b+CD3+TCRαβ- and CD11b+CD3+TCRαβ+. At the moment, the phenotype and function of these subpopulations using human cells has not been assessed.

Methodology: PBMCs were obtained from healthy donors, CD14+ cells were obtained through a positive selection. After 7 days in culture MDM were obtained and characterized by flow cytometry and confocal microscopy. The subpopulations were sorted and cultured for 24h with α-CD3 and α-TNF stimuli. Supernatants were collected to evaluate the profile of cytokines by multiplex. A pleural BCG infection murine model was used to evaluate the role of TNF to favor the presence of TCR+ myeloid cells, three mice phenotypes were used: Wild-type (WT), only expressing the trans-membrane form of TNF (tmTNF-KI) and TNF deficient (TNF-KO).

Findings: Considering CD3 expression, we observed three MDM subpopulations: CD3- (80%) y CD3+ (20%), this last one is divided in CD3+TCRαβ+ (15%) y CD3+TCRαβ-(80%). Both CD3+TCRαβ+ and CD3+TCRαβ- express HLA-I and HLA-II. Moreover, only CD3+TCRαβ+ showed an increase in the expression of CD1 group I family (a, b and c isoforms), CD16 and tmTNF. To clarify if CD3 and tmTNF induce cellular activation, we sorted each macrophage subpopulation and stimulated them with α-CD3 and α-TNF. Our results show that both CD3+ MDM are capable of inducing pro-inflammatory cytokines by CD3/TNF-dependent pathway. However, the secreted profile was subpopulation-specific: CD3+TCRαβ+ MDM produce CCL2, IP10 and Il-1β; whereas CD3+TCRαβ- MDM secrete TNF and IFN-γ. Finally, using a pleural BCG infection murine model, we show that TNF-KO mice are incapable of taking in CD3+TCRαβ+ or CD3+TCRαβ- subpopulations to the site of infection. However, TNF-KI mice were capable of incorporating both subpopulations resembling the WT phenotype; the above suggests an important role of these subpopulations at the site of infection and probably it is tmTNF-mediated.

Conclusion: Here, we demonstrate that a fraction of MDM from healthy human donors express CD3, however only 15% of them is TCRαβ+. This subpopulation (CD3+TCRαβ+) can be activated by CD3/TNF pathway to promote a pro-inflammatory cellular function. Moreover, we demonstrate that tmTNF is sufficient to sustain the cell recruitment of TCRαβ to the site of BCG pleural infection.

REFERENCES

1. Beham, A. W., Puellmann, K., Laird, R., Fuchs, T., Streich, R., Breysach, C., et.al., Kaminski, W. E. (2011). A TNF-regulated recombinatorial macrophage immune receptor implicated in granuloma formation in tuberculosis. PLoS Pathogens, 7(11). https://doi.org/10.1371/journal.ppat.1002375
2. Fuchs, T., Puellmann, K., Hahn, M., Dollt, C., Pechlivanidou, I., Ovsiy, I, et.al., Kaminski, W. E. (2013). A second combinatorial immune receptor in monocytes/macrophages is based on the TCRγδ. Immunobiology, 218(7), 960–968. https://doi.org/10.1016/j.imbio.2012.11.005
3. Puellmann, K., Kaminski, W. E., Vogel, M., Nebe, C. T., Schroeder, J., Wolf, H., & Beham, A. W. (2006). A variable immunoreceptor in a subpopulation of human neutrophils. Proceedings of the National Academy of Sciences of the United States of America, 103(39), 14441–14446. https://doi.org/10.1073/pnas.0603406103

TLR2- dependent sensing of dengue virus induces early RIPK1-mediated necroptosis and TNF-α production

John F Arboleda- Alzate[1,2], Silvio Urcuqui-Inchima[1], Jolanda Smit[2], Izabela Rodenhuis-Zybert[2]

[1]Universidad de Antioquía, Medellín, Colombia
[2]University of Groningen, Groningen, Netherlands
guanojf@gmail.com; silviourcuqui@gmail.com; jolanda.smit@umcg.nl; i.a.rodenhuis-zybert@umcg.nl

Keywords: Dengue Virus, necroptosis, TNF-α, ripk1, TLR2 activation

Disease caused by mosquito-borne dengue viruses is currently one of the most important health concerns worldwide. Clinical outcomes of DENV virus infection vary from a mild self-limited flu-like illness (dengue) to a potentially fatal severe dengue hallmarked by plasma leakage, hypovolemic shock (severe dengue). The severe disease is preceded by an exacerbated secretion of pro-inflammatory and vasoactive mediators such as TNF-α and IL-1β. This excessive cytokine response indicates alterations in the mechanisms controlling the physiological process of inflammation during dengue virus (DENV) infection.

The key to the beneficiary role of inflammation is its regulation. Cell death programs represent key innate mechanisms responsible for tuning the extent and duration of inflammatory responses in course of infection. Indeed, pathogen recognition via different pattern recognition receptors (PRRs) can lead to lytic (necroptosis, pyroptosis) and non-lytic cell death pathways (apoptosis). In general, lytic cell death pathways provide pro-inflammatory signals and thus initiate or prolong process of inflammation while non-lytic, as the case of apoptosis, limits or contract inflammation.

Human peripheral blood monocytes represent one of the most important sentinel cells of the innate immune system. They are responsible for viral sensing, viral degradation and initiation of inflammation. Notably, despite the undeniable significance of responses initiated by monocytes, to date we have no clear view on the cell death mechanisms initiated in course of DENV. In light of the recent discoveries of the effector molecules for necroptosis and pyroptosis we revisited this important issue. Herewith, we elucidated the kinetics and nature of cell death mechanisms induced in the course of DENV infection. Using THP-1 monocytes and human PBMCs as an infection model, we found a biphasic activation of lytic cell death.

An 'early' cell death program was observed within the first 2–6 hours post-infection and did not rely on virus replication whereas a 'late' de novo mechanism occurred 24 hours post-infection and required replicative capacity of the virus. Since, to our knowledge the early lytic program has not been previously described in context of DENV, we first focused on the characterization of this pathway. We found that the early lytic cell death induced by DENV relied on functional RIPK1 and MLKL, two core machinery proteins involved primarily in the execution of necroptosis. Indeed, functional inhibition of RIPK1 and MLKL by necrostatin-1 and necrosulfonamide, respectively decreased levels of the phosphorylated form of MLKL induced in THP-1 cells following exposure to DENV. Interestingly, RIPK1 but not MLKL function was also required for DENV-induced intracellular accumulation of TNF-α at 6 hours post-infection. Finally, the analyses of PRR and internalization-pathway dependency showed that upstream of RIPK1, extracellular recognition of the virus via TLR2 is crucial for both necroptosis and TNF-alpha production. Altogether our data unravel necroptosis as an innate mechanism triggered in cells upon DENV sensing and reveal the dichotomy of TLR2-mediated RIPK1-activation.

Macrophage galactose-type lectin (MGL) receptor plays a critical role in the infection against Trypanosoma cruzi

Tonathiu Rodriguez, Alicia Vazquez-Mendoza, Imelda Juarez-Avelar, Miriam Rodriguez-Sosa

Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico
tonathiurh@hotmail.com; vama_26@yahoo.com.mx; imelda_juarez@yahoo.com; rodriguezm@unam.mx

Keywords: PRRs, T. cruzi, Macrophage galactose-type lectin, immunity response, MGL, galactose moieties

Macrophage galactose-type lectin (MGL) receptor is specific for galactose moieties expressed on the surface of T. cruzi. Chagas disease is caused by the intracellular parasite Trypanosoma cruzi (T. cruzi) which origins cardiomyopathy and digestive affectations. The protective immune response against T.cruzi is characterized by the production of cytokines (TNF-α, IL-12 and IFN-γ) and reactive oxygen species, and oxidative stress. It is unknown about the role immune that play MGL in the function and activation in macrophages (MOs) against T. cruzi infection. This work shows that mMGL by first, multiples functions of MGL in macrophages against the infection of T. cruzi. We found that MOs mMGL-/- infected with T. cruzi in vitro had a greater number of internal parasites than WT MOs. Internal Parasites from MOs mMGL-/- show highter viability. The absence of mMGL provide low production of nitric oxide (NO), reactive oxygen species (ROS), interleukin (IL)-12 and tumour necrosis factor (TNF)-α, associated with lower expression of TLR-2 and TLR-4. Also, MOs mMGL-/- the activation of the molecules such as p38 and NF-κB has been reduced, interestingly ERK-1/2 did not show activation against T. cruzi antigens in MO mMGL-/ -. In addition, mMGL played a key role in MHC-II expression, favouring the activation of antigen-specific cells for T. cruzi infection, producing a greater amount of proinflammatory and anti-inflammatory cytokines in addition to being proliferative.

Dual effects of sags of the EGC operon on innate immune cells

Sofía Noli Truant[1], Belen Sarratea[1], Belen Antonoglou[1], Laura Iannantuono López[1], Carolina Cristina Jancic[2], Emilio Luis Malchiodi[1], Marisa Mariel Fernandez[1]

[1]Universidad de Buenos Aires, Buenos Aires, Argentina
[2]Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina
sofianoli@gmail.com; mbsarratea@hotmail.com; belenantonoglou@hotmail.com; laura_jann@hotmail.com; cjancic@hotmail.com; emalchio@ffyb.uba.ar; mmfernan@ffyb.uba.ar

Keywords: Staphylococcus aureus, Superantigens, IL-10, IL-17, proinflammatory cytokines, CD1a, TNF-α, CD1b, IL-12p40, PBMCs, THP-1 cell, INF-gamma, innate immune response., LT γδ

Bacterial superantigens (SAgs) are enterotoxins that bind to MHC-II and TCR molecules, activating as much as 20% of T cells and promoting a cytokine storm which enhances endotoxic shock and produces immunosuppression, hindering the immune response against bacterial infection. The egc operon reported in Argentinian autochthonous S. aureus strains includes natural variants of SAgs genes seg, sei, sem, sen and seo, each of one appears to present complementary activity upon the immune system.

In the present work, we investigated the effect of SEG, SEI, SEO and SEM on different cells of the innate immune response.

At first, we examined the effect of SAgs on monocytic Thp-1 cells. We observed a wide capacity of the four SAgs to inhibit their proliferation by thymidine: at the maximum concentrations SEO, SEG and SEI inhibited up to 70% and SEM inhibited near 30% ($p<0,0001$). Furthermore, we confirmed the death by flow cytometry, and measured cell death by fluorescent microscopy and nuclear morphology to assess apoptosis and necrosis. Also, we evaluated the production of cytokines. We found a potent production of IL-8, IL-12 and TNF-α by Thp-1 cells after stimulation at 16h and 48h with SAgs, in a concentration dependent manner ($p<0,05$).

That profile was accompanied by the production of IL-12 by PBMCs, but in this case, production of IL-10 was observed, too. Regarding to this, every different SAg had the capacity to stimulate PBMCs to proliferate ($p<0,0001$), but with diverse potency.

Subsequently, we aimed to study if other cells of the innate immunity are responsible for this pro-inflammatory profile. Thus, we evaluated the SAgs effect on human purified γδ T cells from healthy donors by flow cytometry and ELISA. We found that, in contrast to the effect on αβ T cells, only some SAgs activated γδ T cells from 0.1 μM ($p<0.05$) with diverse potency, but became toxic at 10 μM. We also found a significant production of IFN-γ and TNF-α since 0.1 μM ($p<0.05$), with no production of IL-17. Such activity was not related to the αβ TCR binding site, since mutant SAg lacking the ability to bind to this TCR, activated γδ T cells as much as wild type SAgs. Therefore, SAgs display a differential activity on γδ T cells, compared to αβ T cells.

Given that CD1 molecules are targets of γδ TCR we analyzed their expression on human mononuclear cells upon SAgs stimulation, by flow cytometry. We found a differential expression of monocytic CD1a and b in response to these SAgs ($p<0.1$).

Gathered all these results together, we described a M1 profile induced by SAgs on PBMCs and monocytic cells and the capacity of SAgs to stimulate γδ T cells to produce IFN-γ and TNF-α, which strongly suggest an early pro inflammatory activity of SAgs on the innate immune response. In addition, the reduction of the pool of phagocytic and effector cells and the generation of a non-efficient Th1 profile accompanied with anti-inflammatory cytokines as IL-10, would conspire against the successful eradication of an extracellular bacteria.

Searching for the natural ligand of human Aminopeptidase N (CD13)

Georgina Ivette Lopez Cortes, Enrique Ortega Soto

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
gina.lopez.21@gmail.com; ortsoto@biomedicas.unam.mx

Keywords: Monocytes, Signal Transduction, phage display, aminopeptidase N, CD13

Introduction: The Aminopeptidase N (CD13, gp 150, ANPEP) is a membrane protein expressed on different cell types in humans including intestinal and renal epithelial cells, the synaptic membranes of neurons in the Central Nervous System (CNS) and on myelomonocytic cells, such as monocytes, macrophages, dendritic cells and neutrophils. CD13 is a highly glycosylated protein characterized as an enzyme that cleaves N-terminal neutral amino acids from peptides and proteins. CD13 acts also as a viral receptor for human coronavirus and cytomegalovirus, and it is involved in multiple processes such as angiogenesis, tumor cell invasion, cell adhesion and migration (Mina- Osorio, P., 2008), and has been shown to mediate signal transduction events leading to activation of Src, PI3K, FAK and the MAPKs as well as causing cytoskeleton's rearrangements. (Navarrete- Santos, A., et al. 2000, Licona-Limón, I., et al. 2015). In human monocyte-derived macrophages it was shown not only that CD13 crosslinking increases the internalization of particles mediated by known phagocytic receptors (i.e. FcγR's), but also that CD13 can mediate phagocytosis by itself (Licona-Limón, I.,et al. 2015). Because of the many functions in which CD13 participates, it has been considered a moonlighting enzyme (Mina- Osorio, P., 2008). However, the evidence for the abiolity of CD13 to mediate effector functions has been obtained either by crosslinking CD13 with monoclonal antibodies (MoAb), in genetically modified animals or by the use of enzymatic inhibitors, since the natural ligand(s) that could induce those functions have not been identified.

Purpose: The aim of this study is to identify amino acid sequences that could be part of the natural ligand of human CD13.

Methods: The strategy of selecting peptide sequences was accomplished by Phage Display technology over HEK 293 cells transfected to express human CD13/ Aminopeptidase N (Anpep). We used two different libraries: one expressing 12-amino

acid random sequences and another expressing peptides from human brain cDNA. Next, some selected clones were sequenced, and databases were searched for proteins with sequences with similarity to those of the clones.

In order to test whether substrates of the enzyme are able to induce a signal transduction cascade, the activation of Src was quantified in cells incubated with three different substrates of the Aminopeptidase N and an inhibitor of the enzymatic activity., Redistribution of membrane CD13 induced after incubating cells with the same substrates and inhibitor was visualized by fluorescence microscopy.

Results: After three rounds of selection, clones were selected that show a specific affinity to CD13. Individual clones were tested for their specific union to CD13+ cells and not to CD13- cells. The clones with a higher specific binding to CD13+ cells were sequenced and aligned in order to know the peptides with the higher identity values. We found six different peptides that were studied so as to know if their interaction with CD13 could induce the effector functions reported. From those 6 peptides, two were discarded because they resemble a non coding sequence of the mRNA of the peptide and the others sequences are still in consideration.

To evaluate if the substrates could elicit a signal transduction cascade, activation of Src in cells incubated with the substrates was measured by flow cytometry, and the localization of membrane CD13 was visualized by immunofluorescence microscopy. The homogeneous membrane distribution of CD13 did not change in cells incubated with the substrates or with the inhibitor, with few points of CD13 aggregation. In the contrary, aggregation of CD13 in the membrane caused by monoclonal Ab 452 is evident.

REFERENCES

Licona-Limón, I., Garay- Canales, C., Muñoz- Paleta, O. & Ortega- Soto, E., 2015. CD13 mediates phagocitosis in human monocytic cells. Journal of Leukocyte Biology, Volume 98, pp. 85-98.

Mina-Osorio, P., 2008. The moonlighting enzyme CD13 old and new functions to target.. Trends in molecular medicine., 14(8), pp. 361- 371.

Navarrete Santos, A., Langner, J., Herrmann, M. & Riemann, D., 2000. Aminopeptidase N/ CD13 is directly linked to signal transduction pathways in monocytes.. Cellular immunology. , Volume 201, pp. 22-32

TNFR1 modulates the recruitment of CD3+TCRαβ- and CD3+TCRαβ+ myeloid cells to the liver after BCG infection

Leslie Chavez-Galan[1], Valerie Quesniaux[2], Bernhard Ryffel[2], Irene Garcia[3]

[1]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
[2]University of Orléans, Orléans, France
[3]Université de Genève, Geneva, Switzerland
lchavez_galan@iner.gob.mx; quesniaux@cnrs-orleans.fr; bryffel@cnrs-orleans.fr; Irene.garcia-gabay@unige.ch

Keywords: Liver, BCG, TNFR1, inflamation, CD3+ myeloid cells

Background: Tumor necrosis factor (TNF) plays a main role in granuloma formation in response to mycobacterial infection (1). Our previous results have shown that soluble TNF (solTNF) but not transmembrane form (tmTNF), is sufficient to confer liver protection to BCG-induced liver injury (2). Previous data reported the presence of TCR+ myeloid cells in granulomas of patients with tuberculosis and this myeloid subpopulation appears to be regulated by TNF (3). The aim of this study was to explore if TCR+ myeloid cells can be detected in liver granulomas after BCG infection and to define the role of TNF receptors (TNFR1 and TNFR2) in the modulation and maintenance of CD3+ myeloid cells.

Methodology: Liver cells from naïve or 15-days BCG infected mice were analyzed from different mouse strains including: WT, fully deficient for TNFR1 (TNFR1 KO), p55 TNFR1 inactivation in myeloid cells (TNFR1-M KO) or in T lymphocytes (TNFR1-T KO), p75 TNFR2fl/fl mice (TNFR2-Flox), p75 TNFR2 inactivation in myeloid cells (TNFR2-M KO) or in T cells (TNFR2-T KO). Phenotypical characterization was performed by flow cytometry; CD3+ TCRαβ myeloid subpopulations were sorted and evaluated by q-PCR and histology. A murine macrophage cell line was infected in vitro MOI 0.5 and the expression of CD3 evaluated by flow cytometry, western blot and q-PCR.

Findings: Total deletion of TNFR1 affected the myeloid cell recruitment to liver after BCG infection. Considering CD3 expression, we observed in WT mice two CD3+ myeloid subpopulations: CD3+CD11b+TCRαβ+ and CD3+CD11b+TCRαβ- cells.

Specific cell-deficiency of TNFR1 in T cells (TNFR1-T KO mice) resulted in an impaired hepatic CD3+CD11b+TCRαβ- cell subpopulation both in naïve and in infected mice. In contrast, TNFR2 deficiency did not alter CD3+ myeloid cell recruitment to the liver after BCG infection. The expression of CD3 on flow-sorted CD3+ myeloid cells was confirmed by q-PCR and these cells were negative for CD2, a marker of lymphoid cells. Flow-sorted CD3+ cells were physiologically active as they were able to phagocyte mycobacteria and to produce cytokines after re-exposition to BCG.

We also showed that the murine macrophage cell line RAW (4) was able to express the lymphoid marker CD3 after infection with BCG in a dose and time dependent manner. BCG-infected RAW cells upregulate the expression of CD3+ concomitantly to the expression of tmTNF as evaluated by flow cytometry, western blot and q-PCR.

Conclusion: These data suggest that cell-specific deficiency of TNFR1 has also an impact in the presence of myeloid cell subpopulations in response to BCG which is much lower than the one observed when mice are totally deprived of TNFR1. In addition, we observed two myeloid subpopulations defined as CD3+CD11b+TCRαβ- and CD3+CD11b+TCRαβ+, which are able to phagocyte and deliver cytokines, and modulated by TNFR1 during BCG infection. Co-induction of both CD3 and tmTNF following BCG infection together with the importance of TNFR1 suggest a role of CD3 and tmTNF on myeloid cells to activate anti-mycobacterial mechanisms.

REFERENCES

1. Garcia I, Olleros ML, Quesniaux VF, Jacobs M, Allie N, Nedospasov SA, et al. Roles of soluble and membrane TNF and related ligands in mycobacterial infections: effects of selective and non-selective TNF inhibitors during infection. Adv Exp Med Biol. 2011;691:187-201.
2. Olleros ML, Vesin D, Fotio AL, Santiago-Raber ML, Tauzin S, Szymkowski DE, et al. Soluble TNF, but not membrane TNF, is critical in LPS-induced hepatitis. J Hepatol. 2010;53(6):1059-68.
3. Beham AW, Puellmann K, Laird R, Fuchs T, Streich R, Breysach C, et al. A TNF-regulated recombinatorial macrophage immune receptor implicated in granuloma formation in tuberculosis. PLoS Pathog. 2011;7(11):e1002375.
4. Chávez-Galán L, Vesin D, Martinvalet D, Garcia I. Low Dose BCG Infection as a Model for Macrophage Activation Maintaining Cell Viability. J Immunol Res. 2016;2016:4048235.

Intrathecal activation of classical and lectin pathway in patients with Multiple Sclerosis

Alberto Juan Dorta Contreras, Cristobal González-Losada

Universidad de Ciencias Médicas de la Habana, Havana, Cuba
adorta@infomed.sld.cu; chriss.losada@gmail.com

Multiple sclerosis is an autoimmune disease of the central nervous system characterized by chronic inflammation, demyelination, gliosis and neuronal loss; the course can be relapsing-remitting or progressive. MBL, H- and M-ficolins are starters of the lectin pathway.

Keywords: Multiple Sclerosis, Innate immunology, MBL, Intrathecal synthesis, H-ficolin, reibergram, M-ficolin

Objective: To determine if classical and lectin pathway can be synthesized in central nervous system in patients with Multiple Sclerosis.

Materials and Methods: Three patients with diagnosis of Multiple Sclerosis were studied. Serum and cerebrospinal fluid paired samples from were taken for diagnostic purposes with their informed consent. After routine analysis, residual CSF and serum samples were stored at -80°C anonymously and send later to the Central Laboratory of Cerebrospinal Fluid Analysis of the Faculty of Medical Science "Miguel Enríquez" in order to carry out a neuroimmunological analysis.

Albumin and IgG in CSF and serum were quantified by immunochemical nephelometry with kinetic analysis. MBL, H- and M-ficolins were measured by commercial ELISA kits in serum and CSF.

The Reibergram was employed to determine if IgG and MBL can be synthesized in central nervous system or not. Regressions were employed for the evaluation if H- and M-ficolins can be synthesized in central nervous system.

Result: One pediatric patients had a deficiency of MBL in serum (Table 1).

Table 1: Serum and CSF values

Case	Age	MBL in serum ng/ml	MBL in CSF ng/ml	H-ficolin in serum ng/ml	H-ficolin in CSF ng/ml	M-ficolin in serum ng/ml	M-ficolin in CSF ng/ml
1	14	1467	0.7	4758	2.3	1366	8.7
2	18	1	0.1	14272	3.0	7672	0.9
3	42	2582	0.8	22295	3.3	1584	0.6

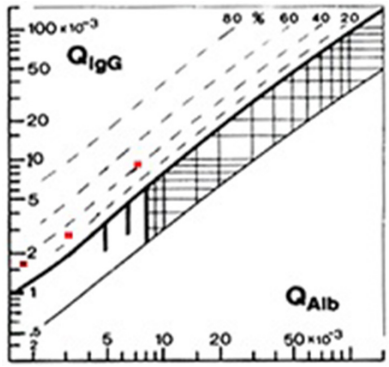


FIGURE 1: IgG reibergram

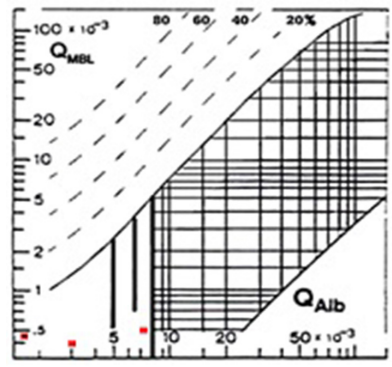


FIGURE 2: MBL reibergram

Every patient had IgG intrathecal synthesis with intrathecal fraction between 22.7% and 42.2 %. One pediatric patient had dysfunction of blood/CSF barrier (Figure 1). Neither of the patients had intrathecal synthesis of MBL (Figure 2).

All patients had intrathecal synthesis of H-ficolin (Figure 3) and M-ficolin (Figure 4).

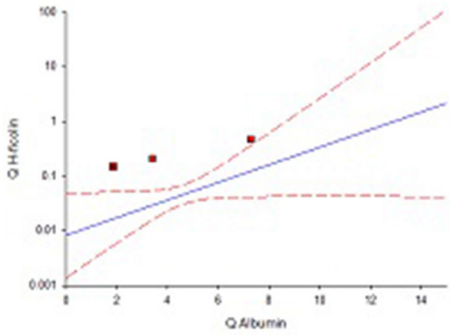


FIGURE 3: Regressions lines. Q H-ficolin vs. Q Albumin

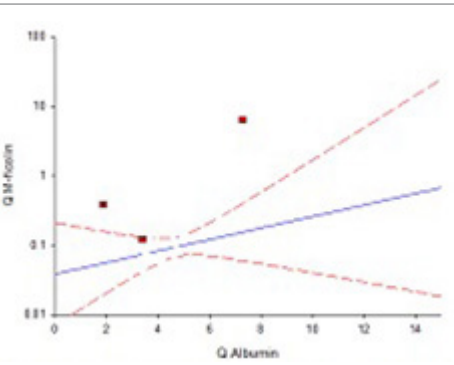


FIGURE 4: Regressions line Q M-ficolin vs. Q albumin

Conclusions: Intrathecal complement activation by the classical and lectin pathway was produced in this Multiple Sclerosis group based on the intratecal synthesis of one or more of the starters or by IgG local synthesis.

Cholera toxin and its non-toxic ß subunit promote the recruitment and training features in skin dentritic cells

Araceli Silvia Tepale-Segura[1,2], Andres Sanchez-Espina[3], Vadim Pérez-Koldenkova[2], Laura Bonifaz[2]

[1]Instituto Politécnico Nacional, Mexico City, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
tepale.araceli@gmail.com; andres.se@live.com; lnma.cmn.sigloxxi@gmail.com; labonifaz@yahoo.com

Keywords: Cholera Toxin, Skin dendritic cells, trained immunity, immflamatory dendritic cells, NON-TOXIC ß SUBUNIT

Introduction: Dendritic cells (DCs) are professional antigen presenting cells that orchestrate immune responses and are the bridge between the innate and adaptive immunity. In steady state, they are characterized by the expression of CD11c and MHC-II. DC activation results in the increased expression of co-stimulatory molecules such as CD40, and CD86 as well as production of pro-inflammatory cytokines such as TNFα. According to the anatomical site where the DCs are located, they can present diverse phenotypes; in the epidermis DCs of Langerhans (CD207+) are found, while in dermis we can find DCs CD11b+ and DCs CD103+. It has been observed in infection models with diverse microorganisms that Leishmania and Listeria, promote the differentiation of monocytes to a DC phenotype named inflammatory dendritic cells (DCs Inf). These are characterized by expression of Ly-6c.

Recent studies have shown that in monocytes treated with B-glucan or C. Albicans an increase in the production of pro-inflammatory cytokines such as TNFα and IL-6 is observed, when these monocytes are challenged with the same or different microorganisms. This mechanism associated to cells from the innate immune system has been called "training". Training is also associated to metabolic changes in the cells once they have been activated. One mechanism by which DCs can be activated is the use of adjuvants. Studies conducted in our group, show that intradermal inoculation (id) with cholera toxin (CT) or its B subunit (CTB) in mice, results in the increase of MHC-II+ cells with an activated phenotype that results in the activation of Th1 or Th17 lymphocytes. However, it is unknown what is the phenotype of the DCs that are recruited to the skin when adjuvants such as CT and CTB are used and if this adjuvant could induce metabolic changes associated with DCs activation and in the development of training.

Aim: To determine the phenotype and activation state of dendritic cells recruited to the skin after inoculation of cholera toxin (CT) and its non-toxic β subunit (CTB) as adjuvants, as well as, to determine if these DCs could develop trained immunity.

Materials and Methods: C57/ BL6 mice were inoculated with 1 μg of CT or 2.5 μg of CTB by intradermal (i.d) immunization in the ear. 3, 7 or 14 days later, skin cells were obtained by enzymatic digestion with collagenase and liberase. The skin dendritic cells were characterized by flow cytometry from cells lineage- (CD3,CD19,TERR119, CD49b), CD45+, MHC-II+, CD103+, CD11b+, Ly6C+, CD86+ TNFα+. Ears were also obtained to perform histological sections and immunofluorescence assays were performed in order to analyze the expression of MHC-II, CD11c, Ly-6c, CD40 and CD86.

Results: Our results show that the i.d. inoculation of CT and CTB not only promotes an increase of both CD11b+ and CD103+ dendritic cells, but also induced their expression of CD86. These results suggest that activated DCs can promote a response of CD4 or CD8 T-cell that is dependent of DC subsets. Interestingly, we also observed the recruitment of DCs Inf (CD11c +, Ly6C +) that express high levels of CD86 compared with conventional subsets. Besides, it was observed that CTB induced expression of TNFα in the CD11b DCs, CD103 DCs and DCs Inf. This results indicate that the CT and CTB could induce important changes in phenotype of the monocyte derived DCs in the absence of an infectious microorganism. Additionally, the MHC-II+ CD11c+ DC infiltrate with an activated phenotype is mainly found in the dermis. In this context, the knowledge of the localization and phenotype of the activated DC subset opens the possibility to modulate the protective immune response. Finally, the re-stimulation with LPS (another ligand of TLRs) in vitro 7 days after CT and CTB inoculation, increased the percentage of TNFα+ cells in DCs CD11b+, DCs CD103+ and DCs inf. This result suggests that DCs in skin are capable of developing training.

Conclusion: Considering that it has been reported that the recruitment of DCs with an inflammatory phenotype, as well as the generation of training, is a determining factor in the induction of a protective immune response, these results are of great relevance in the research for new non-toxic adjuvants for human use in the development of new vaccines.

MAp44 passage from blood to cerebrospinal fluid and the theory of the molecular flow/ cerebrospinal flux

Cristobal González-Losada[1], Inga Zerr[2], Matthias Schmitz[2], Annette Gudmann Hansen[3], Alberto Juan Dorta Contreras[1]

[1]Universidad de Ciencias Médicas de la Habana, Havana, Cuba
[2]Universitätsmedizin Göttingen, Göttingen, Germany
[3]Aarhus University, Aarhus, Denmark
chriss.losada@gmail.com; IngaZerr@med.uni-goettingen.de; matthias.schmitz@med.uni-goettingen.de; ah@biomed.au.dk; adorta@infomed.sld.cu

Keywords: blood-CSF barrier, Lectin pathway, Intrathecal synthesis, MAp44, theory of the molecular flow/ cerebrospinal flux

Introduction: The complement system is activated by three different pathways: classical, alternative and lectin pathway. The lectin pathway, in humans, employs five different humoral starters' molecules: mannan-binding lectin (MBL) [1], ficolins (M-, L-, and H-ficolin; also called ficolin-1, -2, and -3) [2], and collectin (CL-LK) [3]. These starters are binding to serine proteases like MASPs [4] and two non-enzymatic fragments: MAp44 [5] and MAp19 [4].

MAp44, also called MBL/ficolin-associated protein-1 (MAP-1) is a 44 KDa with 361 aminoacids [6]. MAp44 shares its four domains N-terminal with MASP-1 and MASP-3 [5].

MAp44 is an endogenous and natural complement inhibitor of the lectin pathway, displaces MASP-1, 2 and 3 from the MBL complex and significantly inhibits inflammation and complement activation [7]. It decreases C3 deposition and inhibit MBL deposition [8].

Dynamics of its flow from blood to the cerebrospinal fluid was not deeply reported. The normal diffusion of a blood-derived protein from blood to CSF depends of the concentration, molecular size and of other protein characteristics. It could be modulated by the CSF flow rate [9].

MAp44 diffusion from blood to CSF was studied previously [10] but this preliminary results not included controls with large Q albumin.

The aim of this paper is to describe the MAp44 passage from blood to cerebrospinal fluid based on theory of the molecular flow/ cerebrospinal flux.

Materials: 80 CSF and serum samples were obtained for routine analysis, indicated by diagnostic criteria with the informed consent. After it, residual samples were stored at -80°C anonymously, according to the ethics committee of Gottingen University Hospital. The samples were sent later to the Immunology Department of the Aarhus University in Denmark in order to quantify MAp44 and albumin proteins.

It has been selected two groups comprised firstly, 60 control patients without organic brain disease with normal CSF and normal barrier function and secondly, 20 patients without inflammatory diseases but with increased Q Albumin, i.e. with a blood-CSF barrier dysfunction. Control patients were determined non-inflammatory disease to be normal according to clinical and imaging criteria, e.g. headache or non-inflammatory polyneuropathies, and according to their CSF and blood data (normal CSF leukocyte count and protein values), no oligoclonal IgG, age-related normal albumin quotient, normal blood leukocytes and serum C-reactive protein.

Patients with non-inflammatory diseases but with blood-CSF barrier dysfunction (high Q Albumin) as well as all other blood-derived CSF/serum protein quotients (QIgG, QIgA, QIgM) but without any intrathecal major immunoglobulin synthesis. No oligoclonal IgG in CSF was found. This group had spinal canal stenosis, spinal tumor or disc prolapse with normal CSF cell counts and typical findings by electromyography, magnetic resonance and tomography.

Methods: Serum and CSF was stored frozen in aliquots at -80°C until analysis. MAp44 in serum and CSF were measured by a conventional sandwich ELISA technique published previously (5) and albumin by immunochemical nephelometry with kinetic analysis (11)

Result: In Table 1 it has been shown the main serum and CSF MAp44 values. MAp44 values in serum and in CSF has a normal distribution according to Kolgomorov-Smirnov test.The sample coefficient of variation (CV) in CSF was lower than the serum CV. The normal diffusion rate was 1, 02 x 10-5 %, similar as plasminogen with a comparable molecular weight (11).

The regression line indicates a positive variation. An increment of MAp44 concentration in CSF corresponds with an increased Q albumin values (Figure 1).

Table 1: MAp44 and Q Albumin values

	MAp44 CSF ng/ml	MAp44 serum ng/ml	Q MAp44	Q Albumin
Mean	161.70	1610.98	0.14	18.50
CI (95%)	141.20 to 182.20	1468.31 to 1753.63	0.07 to 0.20	8.76 to 28.23
SD	92.13	641.06	0.27	43.74
CV	56.97	39.79		
Kolmogorov Smirnov test	accept Normality (P=0.08)	accept Normality (P=0.22)		

SD: standard deviation; **CI:** confidential intervals; **CV:** population coefficient variation

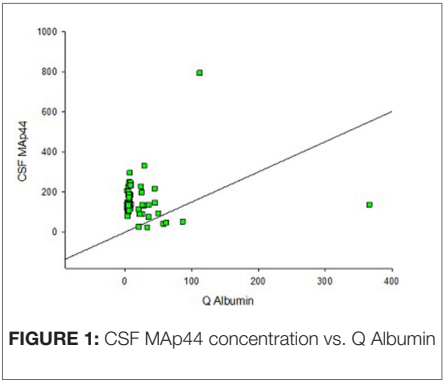


FIGURE 1: CSF MAp44 concentration vs. Q Albumin

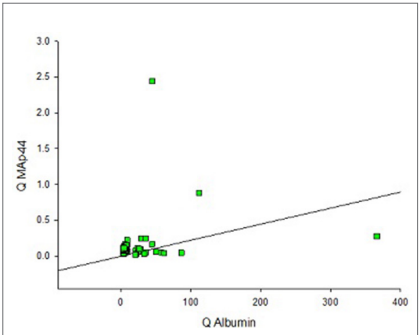


FIGURE 2: Q MAp44 values variation vs. Q Albumin

Figure 2 had a positive slope. It means that an increment of Q MAp44 corresponds with an increased Q albumin values.

Discussion: Mean values of MAp44 in serum in our control differs from other values found in healthy adults [12] with different methods employed.

MAp44 is a blood-derived protein like the majority of the proteins in normal CSF [9] The molecular diffusion/CSF flow model is the base to determine the source of a protein found in CSF, i.e. a blood-derived, or a brain- derived or a leptomeningeal protein.

The levels of MAp44 in CSF are lower than their respective serum samples. It is a strong evidence to catalogue this protein as a blood-derived one.

From the molecular diffusion/CSF model [9, 13, 14] the reduced CSF flow rate is the only mechanism to explain quantitatively the increased concentration of serum proteins in CSF. An increased Q MAp44 was due to the increased diffusion from blood to CSF.

A brain- derived proteins which are released intro ventricular and cisternal CSF are not modified by CSF flow rate i.e. Q Albumin value. A CSF MAp44 value was increasing with the increment of Q Albumin. So, it is another probe that MAp44 is a blood-derived protein mainly.

According to Reiber's postulates [14] about CSF low rate and source-related dynamics of the proteins from blood to CSF, CV value should be smaller in CSF than the corresponding serum.

CV MAp44 in CSF is higher because by the additional individual variation of CSF flow rate. Then MAp44 is a blood-derived protein primarily.

In the case of a blood-derived protein in CSF, an increased coefficient of variation would be expected [13–14]. Therefore, MAp44 in CSF is primarily a blood-derived protein but it is possible to be synthesized in the central nervous system.

MAp44 is a leptomeningeal protein and it should be released along the subarachnoidal spinal flow way. Their rostro-caudal concentration gradient as expected from the theory linearly increasing and it was proven by the evaluation as absolute CSF MAp44 concentration but with its Q albumin reference value.

MAp44 could be synthesized in CSF as well as other components of the complement system.

REFERENCES

1. Héjaa D, Kocsisb A, Dobób J, Szilágyib K, Szászc R, Závodszkyb P, et al. Revised mechanism of complement lectin-pathway activation revealing the role of serine protease MASP-1 as the exclusive activator of MASP-2. PNAS. 2012; 109(26): 10498–10503.
2. Endo Y, Matsushita M, Fujita T. The role of ficolins in the lectin pathway of innate immunity. Int J Biochem Cell Biol. 2011; 43:705–712.
3. Hansen SWK, Ohtanib K, Roy N, Wakamiya N. The collectins CL-L1, CL-K1 and CL-P1, and their roles in complement and innate immunity. Immunobiology 2016, http://dx.doi.org/10.1016/j.imbio.2016.05.012.
4. Thiel S, Jensen L, Degn SE, Nielsen HJ, Gál P et al. Mannan-binding lectin (MBL)-associated serine protease-1 (MASP-1), a serine protease associated with humoral pattern-recognition molecules: normal and acute-phase levels in serum and stoichiometry of lectin pathway components. Clin Exp Immunol 2012; 169: 38-48. Doi:10.1111/j. 1365-2249.2012.04584.x. PubMed: 22670777.

5. Degn SE, Hansen AG, Steffensen R, Jacobsen C, Jensenius JC, Thiel S. MAp44, a human protein associated with pattern recognition molecules of the complement system and regulating the lectin pathway of complement activation. J Immunol. 2009; 183:7371–8.
6. Yongqing T, Drentin N, Duncan RC, Wijeyewickrema LC, Pike RN. Mannose-binding lectin serine proteases and associated proteins of the lectin pathway of complement: Two genes, five proteins and many functions? Biochimica et Biophysica Acta 2012; 1824: 253–262.
7. Gorsuch WB, Chrysanthou E, Schwaeble WJ, Stahl GL. The Complement System in Ischemia-Reperfusion Injuries. Immunobiology. 2012; 217(11): 1026–1033. doi:10.1016/j.imbio.2012.07.024.
8. Pavlov, V.I., Skjoedt, M.O., Siow Tan, Y., Rosbjerg, A., Garred, P., Stahl, G.L. Endogenous and natural complement inhibitor attenuates myocardial injury and arterial thrombogenesis. Circulation 2012; 126: 2227–2235.
9. Reiber H. Knowledge-base for interpretation of cerebrospinal fluid data patterns. Essentials in neurology and psychiatry. Arq Neuropsiquiatr 2016; 74 (6):501-512. DOI: 10.1590/0004-282X20160066.
10. Wildemann B, Oschmann P, Reiber H: Laboratory diagnosis in Neurology. Stuttgart: Thieme; 2010.
11. Dorta-Contreras AJ, Padilla-Docal B, Iglesias González IM, Martínez-Larrarte JP, Castillo-González W, González-Losada C, González-Argote J, Jensenius JC. MAp44: diffusion from blood to cerebrospinal fluid and intrathecal synthesis. The FASEB Journal 2016; 30(1) Supplement 970.1 DOI:10.1096/fj.1530-6860. Disponibleen: http://www.fasebj.org/content/30/1_Supplement/970.1.short
12. Skjoedt MO, Hummelshoj T, Palarasah Y, Hein E, Munthe-Fog L, Koch C, et al. Serum concentration and interaction properties of MBL/ficolin associated protein-1. Immunobiology. 2011; 216:625–632.
13. Dorta-Contreras AJ, Reiber H. Teoría de la difusión molecular/flujo del líquido cefalorraquídeo. Rev Neurol 2004; 39: 564-9
14. Reiber H. Cerebrospinal fluid data compilation and knowledge-based interpretation of bacterial, viral, parasitic, oncological, chronic inflammatory and demyelinating diseases. Diagnostic patterns not to be missed in neurology and psychiatry. Arq Neuropsiquiatr 2016; 74(4):337-350.

Endoreplication in mosquitoes and monocytes is a hallmark in the innate immune memory

Jorge Cime Castillo[1], Rob Arts[2], Valeria Vargas[1], Mihai Netea[2], Salvador Hernández[1], Humberto Lanz-Mendoza[1]

[1]Instituto Nacional de Salud Pública (INSP), Cuernavaca, Mexico
[2]Radboud University Nijmegen, Nijmegen, Netherlands
jor_cime@yahoo.com.mx; rjwarts@gmail.com; valvar21@yahoo.com.mx; Mihai.Netea@radboudum.nl; shernandez@insp.mx; humberto@insp.mx

Keywords: Immunity, Innate, Macrophages, Mosquitoes, endoreplication, trained immunity

Both old and novel studies have demonstrated that innate immunity can adapt upon encountering an infection, a de facto memory. This characteristic has been demonstrated in vertebrates, invertebrates and plants, and has been named trained immunity (TI) or innate immune priming (IP). The molecular mechanism behind TI is not completely understood but in monocytes, epigenetic reprogramming was shown to play a relevant role. Monocytes can be trained by pre-exposure to Candida albicans, β-glucan or BCG vaccine, which results in an enhanced and long-lasting response to microbial components at later time points.

The molecular mechanisms of immune priming or innate immune memory in invertebrates is not known but, recently, some interesting evidence has been obtained. Epigenetic reprogramming is an important mechanism during trained immunity, hence it is necessary to determine the mechanism that allow the cells to rapidly produce sufficient proteins to respond to a second challenge. Other likely mechanisms that may explain properties observed during induction of innate immune memory are DNA synthesis and endoreplication.

Endoreplication refers to multiple rounds of nuclear genome duplications without intervening division/cytokinesis

We previously reported that de novo DNA synthesis occurs in mosquitoes Anopheles albimanus, as shown by the incorporation of bromo-deoxyuridine (BrdU), polytene chromosomes formation, and activation of proliferating cell nuclear antigen (PCNA) in the midgut of these insects after an immune challenge. Such synthesis is most probably by endoreplication, as no mitotic cells have been observed. In a recent study, we documented an enhanced DNA synthesis after the second exposure to the same pathogen

in the midgut of the mosquitoes An. albimanus. Moreover, we explored the role by one of the key factors in cell cycle switching toward endoreplication – hindsight (hnt).

This scenario implies that midgut cells require an increased number of copies of certain genes for a quick and effective response, which may include the fast production of effector transcripts and proteins. This information can be kept in the copies of genes that allow a rapid response to a second insult.

In this study, we analyze the role of the DNA synthesis (endoreplication) in human monocytes and mosquito Anopheles albimanus cell line LSB-AA695BB, during the induction of innate immune memory. We observed that monocytes and mosquito cell line show an intensive DNA synthesis (BrdU incorporation), increasing DNA concentration and hnt overexpression. When DNA synthesis is blocked, the expression of immune memory markers is reduced to control levels.

Inactive dengue virus exposed at the larvae stage induced innate immune memory in Aedes aegypti adult mosquitoes

Valeria Vargas[1,2], Jorge Cime Castillo[2], Humberto Lanz-Mendoza[2]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Nacional de Salud Pública (INSP), Cuernavaca, Mexico
valvar21@yahoo.com.mx; jor_cime@yahoo.com.mx; humberto@insp.mx

Keywords: RNA, Small Interfering, immune memory, Aedes aegypti, Larvae stage, DENV-4

Immune priming (IP) of insects allows them to enhance the effectiveness of the immune response after a previous contact with an elicitor of the immune response. We found innate immune memory against DENV-2 in adult Aedes aegypti, when mosquitoes had a first challenge with psoralen-inactived DENV-2 and subsequently had a second challenge with active DENV-2 (1x107 FFU)/ml), exhibiting a decrease the infection in midgut and carcass, suggesting limited viral replication of DENV-2 (Serrato et al., 2018). However, induction of IP in larvae stage of A. aegypti with inactive virus, could develop innate immune memory in adult mosquitoes against DENV has not been reported. In this study, we demonstrated the induction of IP against DENV-4 in larvae stage, decrease of viral load detected in excrete of individual mosquitoes and follow the infection to 2, 7 and 14 days post-second challenge, without killing the mosquitoes. In the first challenge, larvae were exposed with inactive virus (UV) for 24 hours and then the second challenge were in adult mosquitoes infected with active virus (1x108 PFU/ml). We evaluated immune response after second challenge by analyzing the expression of RNA interference (Argonaute-2, Dicer-2 and R2D2). Results showed that mosquitoes that were exposed from the larval stage with inactive virus (IP), had a greater expression of RNAi after second challenge, compared to mosquitoes that were not previously exposed. Otherwise, mosquitoes that were exposed in larval stage with inactive DENV-4 (IP), showed decrease viral load than mosquitoes that were not exposed to inactive DENV-4. We concluded that the induction of IP with inactive virus in larval stage of A. aegypti, is able to develop an innate immune memory in adult mosquitoes blocking viral replication of DENV.

REFERENCE

Serrato-Salas, J., Hernández-Martínez, S., Martínez-Barnetche, J., Condé, R., Alvarado-Delgado, A., Zumaya-Estrada, F., & Lanz-Mendoza, H. (2018). *De Novo* DNA Synthesis in *Aedes aegypti* Midgut Cells as a Complementary Strategy to Limit Dengue Viral Replication. *Frontiers in Microbiology*, *9*, 801. http://doi.org/10.3389/fmicb.2018.00801

Candida glabrata modulates the intracellular traffic to persist in human osteoblasts

Shantal Lizbeth Baltierra-Uribe, Alfonso Cervantes-Contreras, Nayeli Castrejon Castrejón-Jiménez, Blanca Estela Garcia-Perez

Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

shantal_baliz@hotmail.com; ponchotl@gmail.com; naye_nice85@hotmail.com; abrilestela@hotmail.com

Keywords: Candida glabrata, Osteoblasts, intracellular traffic, rab gtpases, species reactive oxygen

Candida glabrata is the second most frequent cause of candidiasis after Candida albicans, accounting for approximately 15 to 25% of Candida infections. C. glabrata can invade by endocytosis into host cells.

Rab GTPases are critical for intracellular transport following pathogens endocytosis. Several pathogens deploy virulence factors to inhibit their dead by host cells and establish intracellular niches where their growth takes place. The osteoblasts cells are capable to internalize C. glabrata, and the yeast can survive and replicate, but the mechanisms of intracellular transport that C. glabrata exploit are unknown.

In this study we analyzed the trafficking of C. glabrata into osteoblast cell. For that, we evaluate the Rab GTPases involved in intracellular traffic, like Rab5 (early endosomal marker), Rab7 (late endosomal marker) and cathepsin D (to evaluated lysosomal fusion). As cellular model we used the MG-63 osteoblast cell line. Viable and heath-dead reference strain C. glabrata was employed to stimulated osteoblast cells. The MG-63 monolayers were incubated with live and dead yeast, the endosomal trafficking was monitored during 30, 60 and 120 min by immunofluorescence and confocal analysis. Immunofluorescence assays show that viable C. glabrata is not colocalized with Rab GTPases studied. However the dead yeasts were founded colocalized with Rab5, Rab7 and cathepsin D. In other hand we quantified the ROS production in osteoblasts stimulated with viable and dead yeast; both conditions did not induced significant production of this metabolite. These results indicated that viable yeast disrupts endolysosomal maturation suggesting C. glabrata modulated the trafficking to persist intracellularly into osteoblast and this cells can eliminated the dead yeast through lysosomal pathway independently ROS production.

Autophagy contributes to intracellular clearance of Candida glabrata in macrophages

Nayeli Shantal Castrejón-Jiménez[1], Frida Cardozo-Mata[1], Izama Iris Rodríguez-Morua[1], Shantal Lizbeth Baltierra-Uribe[2], Blanca Estela Garcia-Perez[1]

[1]Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[2]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

naye_nice85@hotmail.com; f_rida_24@hotmail.com; izama_jpr@hotmail.com; shantal_baliz@hotmail.com; abrilestela@hotmail.com

Keywords: Autophagy, Candida glabrata, rapamycin, LC3-II, TPH-1

Candida glabrata is considered an opportunistic fungus, together with C. krusei are now emerging as serious hospital acquired infections in immunocompromised patients. Both species are intrinsically resistant to fluconazole and other azoles, and frequency of isolations of these two species has significantly increased recently. It is know that C. glabrata survives and persist intracellularly in macrophages. Autophagy is a process that targets cellular cytoplasmic contents for lysosomal degradation and serves myriad roles in eukaryotic cells. Xenophagy is a type of selective macroautophagy that specifically targets intracellular pathogens to lysosomes, restricting their replication and survival. Autophagy is induced by inanition, hypoxia, rapamycin and TLRs ligands between others. The present study was designed to investigate if the induction of autophagy contributes to elimination of C. glabrata in macrophages. For that, monolayers of macrophages derived of monocytes THP1 cell line were infected by reference strain CBS138 C. glabrata. In some cases monolayers were previously treated with autophagy inductors (inanition, rapamycin and peptidoglycan). Autophagy was evidenced by immunofluorescence stain of LC3-II, a characteristic protein of autophagy. The effect of autophagy induced on C. glabrata elimination was followed by determination of UFC. The results demonstrated that C. glabrata induces the LC3-II expression. When cells were previously stimulated with autophagy inductors, LC3-II protein was visualized in the cytoplasm of the cells, in some cases colocalization of LC3-II with yeasts was notorious. The determination of UCF showed that the three inductors caused a decrease of intracellular yeast; rapamycin was the best stimuli to dead the intracellular yeast. Altogether, the results indicate that autophagy contributes to clearance of intracellular yeast in macrophages and enhance the role of autophagy in the control of intracellular pathogens.

Trichomonas vaginalis DNA modulates the immune response in a murine model of trichomoniasis

Marco Antonio Barajas Mendiola, Laura Edith Castellano Torres, Patricia Cuéllar Mata

University of Guanajuato, Leon, Mexico
barajasmendiola@hotmail.com; laura.castellano@gmail.com; mata@ugto.mx

Keywords: Cytokines, Macrophage Activation, trichomoniasis, immunostimulatory effect, TvDNA

Trichomonas vaginalis (T. vaginalis) is an extracellular flagellated parasite, which colonizes the urogenital tract of humans and is the etiologic agent of trichomoniasis, the most common sexual non-viral transmission disease worldwide (1). Trichomoniasis has been associated with a high risk and susceptibility of acquiring the human immunodeficiency virus (HIV) and human papillomavirus (HPV) as well as risk to develop cervical and prostatic cancer, including adverse outcomes in pregnant women (2–4). Further, this parasite has begun to develop resistance to metronidazole and tinidazole (5). As an extracellular parasite, T. vaginalis needs to adhere to epithelial cells of host´s urogenital tract in order to survive. Hence, the mucosal innate immune system is the first line of defense against of this pathogen. Among the defending cells of the innate immune system, macrophages play an important role in the recognition, control, and clearance of pathogens. Toll-like receptor-9 (TLR-9), a member of the TLRs family, is mainly expressed in the membrane of endosome of macrophages, dendritic cells (DCs), and B lymphocytes (6, 7). TLR-9 recognizes unmethylated CpG dinucleotides (CpG-DNA), which have an important immunomodulatory effect and are common in bacterial and parasitic genomes but rarely present in mammalian genomes (8–12). Therefore, the study of the modulation of the innate immune system represents a valuable alternative to develop strategies to strengthen the host defense against to parasites.

In this work, we studied the stimulatory effect of T. vaginalis DNA (TvDNA) on RAW264.7 macrophages, and its immunomodulatory activity in a murine model of trichomoniasis. The pro-inflamatory activity of high purityTvDNA in RAW264.7 cells was evaluated using a nitric oxide (NO) assay. In presence of TvDNA, RAW264.7 macrophages increased the nitrite production at similar levels as the bacterial lipopolysaccharide (LPS) did, this effect was not observed in RAW264.7 cells stimulated with mammalian DNA or without TvDNA. To investigate the type of response that TvDNA induced in the macrophages, the cytokines mRNA expression was determined by semi-quantitative RT-PCR assays. The TvDNA induced the mRNA expression of pro-inflammatory cytokines IL-6 and IL-12p40 and anti-inflammatory cytokines IL-10 in RAW264.7 cells. Also, in order to deepen in the type of immune response that was

triggered by TvDNA, the macrophage polarization was studied through of ROS production using Nitro Blue Tetrazolium (NBT) assay. When RAW264.7 cells were exposed to TvDNA, induced a high production of ROS at similar levels to those macrophages that were activated with LPS. As it was expected, the production of ROS was not observed in macrophages stimulated with TvDNA when diphenylene iodonium (DPI), a selective inhibitor of NADPH-oxidase (Nox2), was added or when the cells were treated with IL-4 (an anti-inflammatory cytokine). Finally, we evaluated the immunostimulatory effect of TvDNA on a murine model of trichomoniasis. The TvDNA induced a local inflammatory response that promoted the decreased viability of T. vaginalis in infected female BALB/c mice. Our findings suggest that TvDNA can modulate the immune response in favor of host through of the pro-inflammatory mediators, contributing to the elimination of parasite.

REFERENCES

1. Newman L, Rowley J, Vander Hoorn S et al. Global Estimates of the Prevalence and Incidence of Four Curable Sexually Transmitted Infections in 2012 Based on Systematic Review and Global Reporting. PLoS One 2015; 10: e0143304.
2. Ghosh I, Muwonge R, Mittal S et al. Association between high risk human papillomavirus infection and co-infection with Candida spp. and Trichomonas vaginalis in women with cervical premalignant and malignant lesions. J Clin Virol 2017; 87: 43-48.
3. Silver BJ, Guy RJ, Kaldor JM, Jamil MS and Rumbold AR. Trichomonas vaginalis as a cause of perinatal morbidity: a systematic review and meta-analysis. Sex Transm Dis 2014; 41: 369-376.
4. Mielczarek E and Blaszkowska J. Trichomonas vaginalis: pathogenicity and potential role in human reproductive failure. Infection 2016; 44: 447-458.
5. Dunne RL, Dunn LA, Upcroft P, O'Donoghue PJ and Upcroft JA. Drug resistance in the sexually transmitted protozoan Trichomonas vaginalis. Cell Res 2003; 13: 239-249.
6. Pandey S, Kawai T and Akira S. Microbial sensing by Toll-like receptors and intracellular nucleic acid sensors. Cold Spring Harb Perspect Biol 2014; 7: a016246.
7. Latz E, Schoenemeyer A, Visintin A et al. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. Nat Immunol 2004; 5: 190-198.
8. Shoda LK, Kegerreis KA, Suarez CE et al. DNA from protozoan parasites Babesia bovis, Trypanosoma cruzi, and T. brucei is mitogenic for B lymphocytes and stimulates macrophage expression of interleukin-12, tumor necrosis factor alpha, and nitric oxide. Infect Immun 2001; 69: 2162-2171.
9. Krieg AM. CpG motifs in bacterial DNA and their immune effects. Annu Rev Immunol 2002; 20: 709-760.
10. Jones PA and Takai D. The role of DNA methylation in mammalian epigenetics. Science 2001; 293: 1068-1070.
11. Klinman DM, Klaschik S, Sato T and Tross D. CpG oligonucleotides as adjuvants for vaccines targeting infectious diseases. Adv Drug Deliv Rev 2009; 61: 248-255.
12. Das S, Ghosh AK, Singh S, Saha B, Ganguly A and Das P. Unmethylated CpG motifs in the L. donovani DNA regulate TLR9-dependent delay of programmed cell death in macrophages. J Leukoc Biol 2015; 97: 363-378.

Immune response to Trichomonas vaginalis in a murine model of infection

Francisco Javier Rangel[1], Eva Edilia Ávila-Muro[1], Lourdes Andrea Arriaga_Pizano[2], Patricia Cuallar-Mata[1]

[1]*Universidad de Guanajuato, Guanajuato, Mexico*
[2]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
fjrangel_vet@yahoo.com.mx; edilia@ugto.mx; landapi@hotmail.com; mata@ugto.mx

Keywords: Macrophages, Mice, Inbred BALB C, immune response, Polymorphonuclear cells, Tricomonas vaginalis

Trichomonas vaginalis is the most studied member of the Trichomonad species. This parasite is the causal agent of trichomoniasis (1), an important infection of urogenital tract and the most common sexual transmission infection worldwide, arising 275 million of new cases estimated in 2013, and 50% of asymptomatic cases were estimated in North America (2, 3). This infection induces early placental rupture, premature births, and low weight of neonates in pregnant women. Trichomoniasis has been related to an increase in the incidence of cervico-uterine cancer and viral infections such as Human immunodeficient virus (HIV) and Human papilloma virus (HPV) (4–8). The innate immunity is considered the front line against the pathogen initiating by the production of a collection of pro-inflammatory cytokines and the induction of other antimicrobial factors.

There are few studies about the mechanism how immune system cells recruits defense cells and how they mediate the intense inflammatory response present in acute trichomoniasis. An number of polymorphonuclear cells and lymphocytes have been observed in women infected with C. trachomatis, N. gonorrhea, and T. vaginalis (9-11). Also, we know the cytokine profile in serum (12, 13), and a murine intravaginal model has been useful to study the innate response mediated by cytokines in own workgroup (14). However, there are some questions which cannot be answered in this model. Then, we established a model of peritoneal infection to discern the role of each cellular population participating along with the infection. This will allow us to know the impact of the different immune cellular types on the immunomodulation induced by the parasite or its clearance.

The aim of this work was to determine the kinetic of the different innate cell population that arrived to the infection site in an intraperitoneal murine model. This required analyses of broad pathologic damages, systemic changes, clinical signs induced in the mice, parasite viability and inoculum size to gain an intense immune response but not

animal death. The model allowed us to study the innate immune response with some advantages such as: peritoneum free of another microorganism, unnecessary estral cycle affecting maybe the infection establishment, and abundant sample quantity and easy for handling. In this model, we carried immunophenotyping of the immune innate cells attending the peritoneum at different times of infection. Peritoneal washes were performed, and cells were labeled with a monoclonal antibody cocktail against the clusters of differentiation (CD3, C19, Ly-6C and F4/80) to immunophenotyping, and propidium iodide (PI) to determine cell viability. Later, the determination of different cell populations was analyzed by FACs. In early infection, polymorphonuclear cells and monocytes were the most abundant populations in the immune response to T. vaginalis in our murine experimental infection model (41% and 32%, respectively). Those cells populations were replaced by inflammatory macrophages (52%) at nine days post-infection. We are working to define the main role developed by each immune cell type and its impact on the parasite viability. These findings suggest that the peritoneal infection can guide us to a major understanding about role of immune cells in vaginal trichomoniasis and its impact in clearance of the parasite.

REFERENCES

1. Petrin D, Delgaty K, Bhatt R, Garber G. Clinical and microbiological aspects of Trichomonas vaginalis. Clin Microbiol Rev. 1998;11(2):300-17.
2. Mielczarek E, Blaszkowska J. Trichomonas vaginalis: pathogenicity and potential role in human reproductive failure. Infection. 2015.
3. Twu O, de Miguel N, Lustig G, Stevens GC, Vashisht AA, Wohlschlegel JA, et al. Trichomonas vaginalis exosomes deliver cargo to host cells and mediate host:parasite interactions. PLoS Pathog. 2013;9(7):e1003482.
4. Kharsany AB, Hoosen AA, Moodley J, Bagaratee J, Gouws E. The association between sexually transmitted pathogens and cervical intra-epithelial neoplasia in a developing community. Genitourin Med. 1993;69(5):357-60.
5. McClelland RS, Sangare L, Hassan WM, Lavreys L, Mandaliya K, Kiarie J, et al. Infection with Trichomonas vaginalis increases the risk of HIV-1 acquisition. J Infect Dis. 2007;195(5):698-702.
6. Smith JD, Garber GE. Trichomonas vaginalis infection induces vaginal CD4+ T-cell infiltration in a mouse model: a vaccine strategy to reduce vaginal infection and HIV transmission. J Infect Dis. 2015;212(2):285-93.
7. Yow MA, Tabrizi SN, Severi G, Bolton DM, Pedersen J, Longano A, et al. Detection of infectious organisms in archival prostate cancer tissues. BMC Cancer. 2014;14:579.
8. Kissinger P, Mena L, Levison J, Clark RA, Gatski M, Henderson H, et al. A randomized treatment trial: single versus 7-day dose of metronidazole for the treatment of Trichomonas vaginalis among HIV-infected women. J Acquir Immune Defic Syndr. 2010;55(5):565-71.
9. Cauci S, Culhane JF. Modulation of vaginal immune response among pregnant women with bacterial vaginosis by Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoeae, and yeast. Am J Obstet Gynecol. 2007;196(2):133.e1-7.
10. Fichorova RN. Impact of T. vaginalis infection on innate immune responses and reproductive outcome. J Reprod Immunol. 2009;83(1-2):185-9.

11. Reighard SD, Sweet RL, Vicetti Miguel C, Vicetti Miguel RD, Chivukula M, Krishnamurti U, et al. Endometrial leukocyte subpopulations associated with Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis genital tract infection. Am J Obstet Gynecol. 2011;205(4):324.e1-7.
12. Yadav M, Gupta I, Malla N. Kinetics of immunoglobulin G, M, A and IgG subclass responses in experimental intravaginal trichomoniasis: prominence of IgG1 response. Parasite Immunol. 2005;27(12):461-7.
13. Malla N, Yadav M, Gupta I. Kinetics of serum and local cytokine profile in experimental intravaginal trichomoniasis induced with Trichomonas vaginalis isolates from symptomatic and asymptomatic women. Parasite Immunol. 2007;29(2):101-5.
14. Olmos-Ortiz LM, Barajas-Mendiola MA, Barrios-Rodiles M, Castellano LE, Arias-Negrete S, Avila EE, et al. Trichomonas vaginalis exosome-like vesicles modify the cytokine profile and reduce inflammation in parasite-infected mice. Parasite Immunol. 2017;39(6).

Trichomonas vaginalis DNA modifies microbiota in BALB/c female mice infected with the parasite

Elba Andrea Falcon[1], Marco Antonio Barajas Mendiola[1], Laura Edith Castellano-Torres[2], Paticia Cuellar-Mata[1]

[1]Universidad de Guanajuato, Guanajuato, Mexico
[2]University of Guanajuato, Leon, Mexico
eacompanera@gmail.com; barajasmendiola@hotmail.com; laura.castellano@gmail.com; mata@ugto.mx

Keywords: Trichomonas vaginalis, Vaginal Smears, Balb/c mice, vaginal microbiota, TvDNA

Trichomoniasis is the most common sexual non-viral transmission disease worldwide. A prevalence of 100,988,000 cases and an incidence of 143 million cases were reported in 2012.This disease has been associated with a high risk to acquire Human Immunodeficiency virus (HIV),Human Papillomavirus (HPV),and cervical and prostatic cancer. Nitroimidazole derivates are efficient drugs against Trichomonas vaginalis (Tv), the responsible pathogen, however some clinical isolates has acquired mechanisms of resistance to these drugs. It is necessary to develop new strategies in order to reduce the incidence and prevalence of trichomoniasis.

Our workgroup has been studied the effect of Trichomonas vaginalis DNA (TvDNA) on the innate immune response. Our previous results showed that TvDNA activated RAW264.7 macrophages inducing the nitric oxide (NO)production. Also, TvDNA induced the expression of pro-inflammatory cytokines IL-6 and IL-12p40 and anti-inflammatory cytokines IL-10 in a TLR9-dependent manner. Moreover, TvDNA pre-treatment of infected female mice reduces the viability of the parasite; nevertheless, the mechanism of these effects has not been clarified.

In this work, we analyzed the changes on the major bacterial genders in the vaginal microbiota of infected female mice pre-treated with TvDNA, by microbiological and semi quantitative PCR assays using vaginal washes. Because it has been reported that the microbiota in the vaginal area plays an important role through several homeostatic functions. An alteration in the prevalent bacterial genders could impact in the immunoresponse

We observed a decrease in Lactobacillus spp.(Lac)and Staphylococcus spp(Sta) population up 80–90%in female mice infected with Tv, while Streptococcus spp (Str) population increased approximately 23 times at 14 days post-infection (dpi). Female

mice treated only with TvDNA, showed an increase in the Lac population (10 times) and Sta population (6 times), at 10 and 14 dpi, respectively. Sta population decreased to initial values on this period, while the Str population increased 20 times at 8 dpi. Interestingly, when the infected female mice were pre-treated with TvDNA, the Lac remained practically constant until 14 dpi, while both the Sta and the Str population decreased almost totally at 10 dpi and 4 dpi, respectively. Additionally, the PCR assays confirmed the presence of the Lac and Str in all the conditions but the presence of Str was observed only at 10 and 14 dpi in TvDNA pre- treated and untreated mice infected with Tv (4, 8, 10, and 14 dpi). These results suggest that TvDNA could influence the main bacterial population in the vaginal microbiota, affecting the viability of Tv in benefit of the host.

MIF gene polymorphisms are not a genetic risk factor for NSV susceptibility in a Western Mexican population

Alejandra Garcia-Orozco[1,2], Itzel Citlalli Alcazar-Rios[2], José Francisco Muñoz-Valle[3,4], Anabell Alvarado-Navarro[2,3], Ricardo Quiñones-Venegas[5], Mary Fafutis-Morris[2,3]

[1]Doctorado en Ciencias Biomédicas, Con Orientaciones en Inmunología y Neurociencias, Universidad de Guadalajara, Guadalajara, Mexico
[2]Centro de Investgación en Inmunología y Dermatología (CIINDE) /Instituto Dermatológico de Jalisco "Dr. José Barba Rubio", Zapopan, Mexico
[3]Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
[4]Instituto de Investigación en Ciencias Biomédicas (IICB), Guadalajara, Mexico
[5]Insituto Dermatologico de Jalisco 'Dr. José Baba Rubio', Zapopan, Mexico
alejandra.garcia.orozco@gmail.com; citlalli.air@gmail.com; biologiamolecular@hotmail.com; bell2000_mx@yahoo.com; ricardoquiv@gmail.com; mfafutis@gmail.com

Keywords: Vitiligo, susceptibility factors, Macrophage Migration Inhibitory Factor (MIF), Non-segmental vitiligo, MIF gene polymorphisms

Vitiligo is a depigmenting disorder of the skin, characterized by achromic macules caused by a selective loss of melanocytes. Its etiopathogenesis is unclear, but it has been associated with autoimmune processes. Macrophage migration inhibitory factor (MIF) is an immunoregulatory cytokine associated with autoimmune inflammatory diseases. Two polymorphisms identified in the promoter region has been associated with increased plasma levels of MIF and with an increased risk to develop autoimmune diseases.

In this case-control study, we investigated the association of MIF promoter polymorphisms with NSV and MIF serum levels in a Western Mexican population. Genotyping of the -794CATT5-8 and -173 G>C MIF polymorphisms was performed by PCR and PCR-RFLP respectively in 94 NSV patients and 103 healthy subjects. MIF serum levels were determined by ELISA.

For STR −794 CATT5-8 MIF a significant increase of MIF (genotypes without the risk allele: 5.57 vs. genotypes with allele risk: 4.40 ng/mL; $p<0.001$) levels was found in NSV patients when they compared according to a genetic model (Figure 1). For the SNP -173 G>C MIF a slight increase of MIF (GG: 4.86 vs. GC+CC: 4.57 ng/mL; $p=0.09$), this difference was not significant (Figure 1). The distribution of STR −794 CATT5-8 and SNP -173 G>C MIF genotypes and allele frequencies in NSV patients did not differ

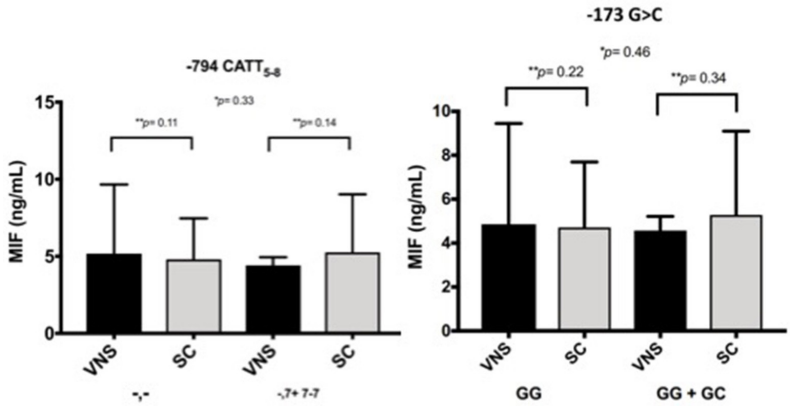


FIGURE 1: Concentrations of MIF by genotypes of –794 $CATT_{5-8}$ –173 G>C *MIF* according to the genetic model of dominant inheritance. a) Concentrations of MIF for genotypes of –794 $CATT_{5-8}$ b) Concentrations of MIF for genotypes of –173 G> C; (-. - = genotypes without the risk allele: -. 7 = heterozygous genotypes with the risk allele) G: guanine; C: cytosine; ** Test Kruskal-Wallis ** U Mann-Whitney considering a level of significance of p <0.05.

from that in healthy subjects (p>0.05). Moreover, there was no association according to a genetic model with susceptibility to NSV. In conclusion, the -794CATT5-8 and -173 G>C polymorphisms of the MIF gene does not confer risk for NSV susceptibility in a Western Mexican population.

Distribution of KIR3DL1/S1 Natural Killer Cell Immunoglobulin like Receptors (KIR) and their HLA Class I ligands in Mestizos from Mexico City

Betsy Gonzalez-Q[1,2], Guadalupe Sanchez[2], Andrea Munguía[1], Miriam Valencia[1], Hilario Flores[1,2], Clara Gorodezky[1,2]

[1]Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), México, Mexico
[2]Fundación Comparte Vida, A.C., Mexico City, Mexico
bgonzalez@fundacioncompartevida.org.mx; rosasenoctubre@yahoo.com; orionica@yahoo.com; mivama@yahoo.com.mx; hilario.flores@salud.gob.mx; clarag@unam.mx

Keywords: innate immunity, KIR genes, Population Immunogenetics, KIR3DL1/S1, HLA ligands of KIR

Introduction: It is well known that KIR3DL1 and KIR3DS1 segregate as alleles of the same locus. Random combination of KIR3DL1-3DS1 and HLA-A or B ligands has vast potential for varying the human NK cell response to infection, autoimmunity and transplantation. Two lineages of inhibitory 3DL1 allotypes and one of activating 3DS1 evolved under balancing selection. To be able to get an insight on the role of innate immunity in different diseases and transplantation, it is relevant to explore first, the allele frequency (AF) in healthy individuals, since they vary among populations. Thus, we sequenced the KIR3DL1-S1 gene and we correlated with their Bw4, HLA-A* and HLA-B* ligands, in healthy Mexicans Mestizos, to be able to further analyze it in different pathologies.

Methods: KIR3DL1-S1 was typed by SBT in 100 unrelated Mestizos residents of Mexico City. The X age was 35 y and 65% were females. KIR exons 1, 2, 3, 4, 5, and 7–9, were sequenced in both directions with primers kindly provided by Dr. P. J. Norman. SBT was performed using a 3500 Sequencer. KIR allele typing was analyzed with the Assign SBT v4.7.1 software. HLA typing was done by a Luminex SSOP and SBT techniques, and the analysis was performed with the Matchit DNA v1.2.0 software. KIR Allele Frequencies and HLA ligands were obtained by direct counting.

Results: KIR3DL1 was found in 97% of the subjects and 42% carried 3DS1. HLA-A/B Bw4 ligand was present in 63% and 49% carry HLA-B* alleles that are Bw4. We found 19 3DL1-S1 alleles, and interestingly a novel allele for KIR3DL1*00401 with a single difference in exon 1 at position 5C>T. The most frequent alleles were 3DL1*01502

(23%), 3DS1*01301 (20%), 3DL1*00101 (13%), 3DL1*00501 (11%). The most frequent allele combinations were 3DL1*01502-3DS1*01301 (12%) and 3DL1*01502-3DL1*00101 (10%).

Conclusions: This is the first study done by SBT of 3DL1/S1 in Mexican Mestizos. Four alleles showed AF over 10%: 3 were 3DL1: *01502, *00101 & *00501 and one was 3DS1: *01301. We also discovered a novel 3DL1 allele; 00401new. KIR gene frequency is in concordance with two previously published data in Mexicans by Gorodezky and her group, were 3DS1 was present in 40.7% and 42% and 3DL1 in 100% and 99% respectively. Our results differ from those in Hispanics, in 17/ 25 alleles, although they share AF similitudes in the most frequent ; 3DL1*01502, 3DL1*00101, 3DL1*00501 and 3DS1*01301. These results emphasize the relevance of analyzing the genetics of populations taaking into account the ethnicity of the group. 3DL1*01502, 3DL1*00501 and 3DS1*01301 represent the ancient lineages and together account for around 50% of all alleles, as shown by the 28 populations analyzed by P Norman et. Al., while KIR3DL1*001 is a recombinant allele of the D0 from *005 (differs at p182 and 283) and the D1–D2 from *015 (differs at p47 and 54). Our data differ from the Warao, Yucpa and Bari South American Indians from Venezuela, since they carry only between 4 & 6 alleles, being 3DS1*01301 & *1502 the most common ones, demonstrating the KIR allele pattern restriction in Amerindians. The knowledge of KIR3DL1-S1 and its HLA ligand combinations is essential, since the stronger the inhibition of an NK cell to attack a healthy cell, its response towards unhealthy cells is stronger. Thus, natural selection for the more inhibitory forms of 3DL1 reflects selection for NKs with stronger response capabilities to attack infections. KIR-HLA interactions of the polymorphic 3DL1 allotypes with HLA molecules presenting almost limitless peptide variants, are central to NK innate immunity function and for understanding the role that these interactions play in health and disease. This knowledge will allow identifying potential areas for therapeutic intervention in different clinical areas, such as transplantation, autoimmune disease and infections. Therefore, studies on KIR allele diversity are crucial.

Neuroimmunological response of MBL, H- and M-ficolins in patients with Guillain-Barré syndrome

Cristobal González-Losada, Alberto Juan Dorta Contreras

Universidad de Ciencias Médicas de la Habana, Havana, Cuba
chriss.losada@gmail.com; adorta@infomed.sld.cu

Guillain-Barré syndrome is an acute autoimmune polyradiculoneuropathy. Lectin pathway can by activated by starters like MBL, H- and M-ficolins. These starters are binding to MASPs protein in order to activate the enzymatic cascade of the complement system.

Keywords: Guillain-Barré syndrome, Lectin pathway, MBL, H-ficolin, M-ficolin

Objective: To determine if MBL, H- and M-ficolins can be synthesized in central nervous system in patients suffering from Guillain-Barré syndrome.

Materials and Methods: Retrospectively were studied patients due to Guillain Barre Syndrome of many etiology.

Five CSF and serum paired samples were taken from patients due to Guillain Barre Syndrome of different hospitals of province La Habana, Cuba. All samples were taken for routine analysis, indicated by diagnostic criteria with the informed consent of the patients. After routine analysis, residual CSF and serum samples were stored at -80°C anonymously and send later to the Central Laboratory of Cerebrospinal Fluid Analysis of the Faculty of Medical Science "Miguel Enríquez" in order to carry out a neuroimmunological analysis.

We selected two groups for this study: one adult patients older than 21 years old and four pediatric patients older than 3 years old and younger than 18 years old.

CSF and serum albumin were quantified by immunochemical nephelometry with kinetic analysis. MBL, H- and M-ficolins were measured by commercial ELISA kits published previously.

The Reibergram was employed in order to determine if MBL can be synthesized in central nervous system or not. Regressions were employed to evaluate if H- and M-ficolins can be synthesized intrathecally.

Result: One adult patient has an early stage HIV-1 infection. Three pediatric patients had respiratory symptoms associated to Mycoplasma pneumoniae infection two weeks ago. The other pediatric case had gastrointestinal symptoms associated to Campylobacter jejuni infection in the previous week.

All patients exhibit normal values of MBL, H- and M-ficolins in serum and CSF (table 1).

All patients had dysfunction of blood-CSF barrier. One pediatric patient and adult patient do not had intrathecal synthesis of MBL (figure 1).

Table 1: Serum and CSF values

Case	Age	Serum Albumin g/l	CSF Albumin mg/ml	Serum MBL ng/ml	CSF MBL ng/ml	Serum M-ficolin ng/ml	CSF M-ficolin ng/ml	Serum H-ficolin ng/ml	CSF H-ficolin ng/ml
1	6	39.94	206.5	1488	4.5	1369	0.2	41447	84.8
2	55	14.13	109.6	188	0.0	756	0.1	25267	19.1
3	6	87.7	450.3	1523	12.5	556	0.8	58121	283.3
4	12	69.4	974.59	783	11.9	767	0.3	35090	426.0
5	17	77	629.63	716	2.9	1001	0.8	26986	116.0

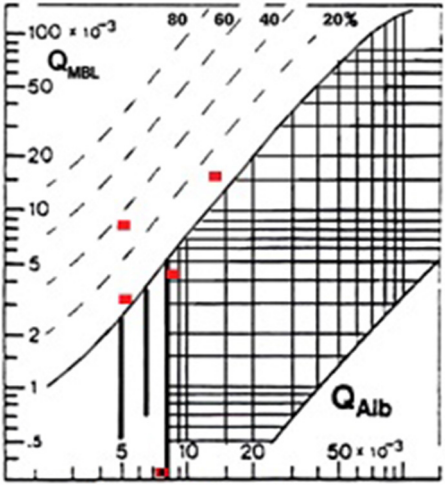


FIGURE 1: MBL reibergram

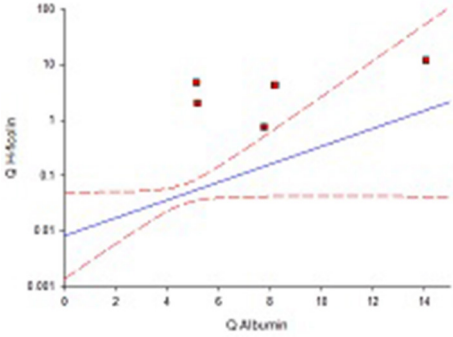


FIGURE 2: Regressions lines. Q H-ficolin vs. Q albumin

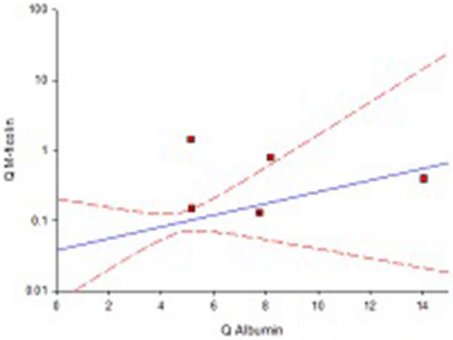


FIGURE 3: Regressions line Q M-ficolin vs. Q albumin

All patients had H-ficolin intrathecal synthesis (Figure 2), but one pediatric patient and adult patient do not had intrathecal synthesis of M-ficolin (Figure 3).

Conclusions: Intratecal activation of the lectin pathway is possible in patients with Guillain-Barré syndrome.

Myosin 1F promotes macrophage pro-inflammatory phenotype during colitis by controlling AKT/STAT signaling

Zayda Lizbeth Piedra-Quintero[1], Angélica Silva-Olivares[1], Raúl Bonilla-Moreno[1], José Luis Maravillas-Montero[2], Mineko Shibayama[1], Nicolás Villegas-Sepúlveda[1], Oscar Medina-Contreras[3], Leopoldo Santos-Argumedo[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[3]Hospital Infantil de México Federico Gómez, Mexico City, Mexico
zpiedra@cinvestav.mx; angelica0517@yahoo.com; rbonilla@cinvestav.mx; maravillas@cic.unam.mx; mineko@cinvestav.mx; nvillega@cinvestav.mx; omedina@himfg.edu.mx; lesantos@cinvestav.mx

Keywords: Colitis, Inflammation, Macrophages, Myosin 1F, AKT/STAT signalin

Introduction: Macrophages are a heterogeneous population of myeloid cells characterized by their ability to phagocyte, process and present antigens, as well as regulate chemokine and cytokine dependent inflammatory responses. Under homeostatic conditions, macrophages release anti-inflammatory mediators to maintain the surrounding tissue under anergic conditions. However, during inflammation the monocytes recruited from bone marrow to inflamed sites differentiate into macrophages. These macrophages activate immune cells or stimulate their differentiation through the secretion of proinflammatory cytokines such as: IL-1β, IL-6, IFN type I and TNFα. Recently, it was reported that AKT and STAT signaling is required to induce the differentiation of macrophages to a pro-inflammatory phenotype. The broad set of inflammatory mediators released by macrophages, however can also exacerbate epithelial damage in mucosal tissue. Thus, understanding the signaling pathway(s) involved in macrophages polarization to a proinflammatory phenotype, has acquired relevance in the development of therapies aimed to control inflammatory diseases, such as colitis. In immune cells, it is well known that cytoskeleton proteins such as myosins are involved in the trafficking and secretion of cytokines. Myosin 1F (Myo1F), a class I myosin that is expressed a significant level as mRNA in myeloid cells has been involved in the regulation of motility in neutrophils. However, its role in macrophage function is unknown.

Objective: To evaluate the role of Myo1F in colonic macrophages polarization to a pro-inflammatory phenotype.

Materials and Methods: A mice model of colitis induced by epithelial damage induced with DSS, was carried out in WT and Myo1F-/- mice in order to evaluate the contribution of the myosin 1F in development of colonic inflammation. Damage was evaluated by Hematoxylin and Eosin staining and immunophenotypification of the immune cell populations at the colon was performed by multi-color flow cytometry. ELISA assay was employed to determine the cytokine release. AKT and STAT activation status was analyzed by western blot in WT and Myo1F-/- bone marrow-derived macrophages (BMM) or macrophage cell lines transduced with Myo1F-GFP. Subcellular localization of Myosin 1F, p-STAT and macrophage markers were carried out by confocal microscopy.

Results: In vitro we observed that myosin 1F protein was absent in monocytes but it was highly expressed in macrophages. Also using an acute model of colitis, we observed an increase in myosin 1F protein levels in the colonic epithelium of WT animals. Additionally, myosin 1F-deficeint mice displayed a less severe disease when compared to WT mice, marked by a decrease in cellular infiltration, ulcers, hemorrhages and reduced epithelial damage. In addition, we observed a decrease in IL-6 and, most importantly, in IL-1β secretion in colonic explants obtained from myosin 1F deficient mice treated with DSS when compared with WT animals. Using an in vitro model we demonstrated that Myo1F regulates IL-1β production and secretion via AKT and STAT signaling. In agreement with these findings, decreased levels of p-AKT(S473), p-STAT1(S727), p-STAT1(Y701) and pSTAT3 were observed in BMM Myo1F-/- stimulated with LPS or with the IFN-γ/LPS. Furthermore, during colitis AKT and STAT phosphorylation was reduced in the colonic mucosa of Myo1F knock out mice when compared with WT.

Conclusion: Myosin 1F enhances AKT and STAT activation in macrophages during inflammation and regulates cytokines secretion.

Cathepsin L3 from Fasciola hepatica induces IL-1β and IL-18 secretion in a NLRP3 dependent manner on dendritic cells

Daiana Pamela Celias[1,2], Leonardo Micael Silvane[1,2], Ileana Corvo[3,4], Manuel Fresno[5], Alicia Arranz[5], César Pruzzo[6], Rodrigo Sanabria[6,7], Laura Cervi[1,2]

[1]National University of Cordoba, Córdoba, Argentina
[2]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI CONICET), Cordoba, Argentina
[3]University of the Republic, Montevideo, Uruguay
[4]Centro Universitario Regional Litoral Norte Paysandu, Universidad de la Republica, Paysandu, Uruguay
[5]Centro de Biología Molecular Severo Ochoa (CSIC), Madrid, Spain
[6]National University of La Plata, La Plata, Argentina
[7]Instituto de Investigaciones Biotecnológicas (IIB-INTECH), Chascomús, Argentina
daiana.celias@hotmail.com; lsilvane@fcq.unc.edu.ar; ilecorvo@gmail.com; mfresno@cbm.csic.es; aarranz@cbm.csic.es; cesari_pruzzo@hotmail.com; sanabriarodrigo@hotmail.com; lcervi@fcq.unc.edu.ar

Keywords: Caspases, Dendritic Cells, Fasciola hepatica, IL-1β, NLRP3, Inflammasome, IL-18, Cathepsin L3

Cathepsin L3 (CL3) is a cystein protease with collagenolytic activity, highly expressed in the juvenile larval stage of the helminth parasite Fasciola hepatica. Its ability to degrade collagen might facilitate the migration of parasite through the host tissues. However, there is no information about its interaction with the immune system. On the other hand, numerous helminth-derived molecules have been described that are able to modulate dendritic cells (DC) activity, which in turn are capable to polarize the adaptive immune response2. Furthermore, several studies have suggested that inflammasome, a multiprotein oligomer that is one of the components of the innate immune system, could be involved in these effects 3,4,5,6. The NLRP3 inflammasome is formed by a set of cytosolic proteins: a receptor (Nod)-like receptor family pyrin domain containing 3 (NLRP3), the adaptor protein apoptosis-associated speck-like protein (ASC), and the pro-caspase-1. Upon stimulations, NLRP3 inflammasome components are assembled leading to the activation of caspase-1, which is responsible for the maturation of pro-inflammatory cytokines, such as bioactive interleukin-1β (IL-1β) and interleukin-18 (IL-18) 7. Taking into consideration all above, the aim of this work was to study the ability of CL3 to modulate the inflammasome activation in bone marrow-derived DC.

DC were prepared by culturing bone marrow cells isolated from femurs of C57BL/6, Caspase-1/11KO or NLRP3KO mice in complete RPMI 1640 with GM-CSF from J558 cell line supernatant. To activate the DC, cells were cultured with a recombinant

CL3 (20 μg/ml) or rvCL3 (variant inactive of CL3), (20 μg/m), both produced in Hansenula polymorpha expression system and subjected to an endotoxin removal with polymyxin columns; or with LPS (1 ug/ml) and ATP (5 mM). Cell viability was assessed by adding a MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), incubating the cells for 4h at 37°C and absorbance was read at 570 nm. Optical density of DC treated with medium alone was considered as 100% cell viability. IL-1β and IL-18 secretion were quantified in culture supernatants by ELISA. DC from C57BL/6, Caspase-1/11KO or NLRP3KO mice were then cultured with allogeneic splenocytes from BALB/c mice during 5 days and IL-4, IL-13 and IFN-γ were measurement in supernatants. Besides, CL3 (previously labeled with Alexa Fluor® 488 Microscale Protein Labeling Kit) was added to DC preparations, and incubated for 30 minutes or 4 hours. Next, the cells were stained with EEA-1 (early endosomes) and LAMP (lysosomes), examined with an Olympus FV1200 confocal microscope and images were analyzed using ImageJ software. Further cell lysates (treated or not with CL3 or LPS as control) were analyzed by western blot to evaluated IκBα. On the other hand, a pro-IL-1β mouse recombinant, was incubated with CL3 or rvCL3 at different concentrations for 4 h and IL-1β levels were quantified by ELISA.

Cell viability of DC treated with CL3, was not affected and significant amounts of IL-1β and IL-18 were detected in culture supernatants ($p<0.05$). While, DC cultured with rvCL3, did not produce IL-1β or IL-18, indicating that the enzymatic activity of CL3 could be involved in this effect. Other cytokines measured were not found.

In order to know whether CL3 is taken by DC and located in some intracellular compartment or simply remained and fulfilled its role from the extracellular medium, experiments using confocal microscopy were performed. We determine that labeled CL3 is internalized by DC, with a weak colocalization to EEA and LAMP positive compartment being mostly targeted to the cytosol.

On the other hand, we investigated whether IL-1β and IL-18 secretion induced by CL3 in DC cultures, could be enhanced by the addition of a first or second signal as LPS or ATP, respectively. We observed that CL3 in combination with LPS or ATP, did not promote a higher IL-1β and IL-18 secretion. Therefore, CL3 alone would be triggering the necessary mechanisms for IL-1β and IL-18 production. Then we studied whether CL3 favored NF-κB activation by western blot. We did not observe IκBα degradation, which indicate NF-κB activation, when DC were cultured with CL3, unlike the control, LPS-treated DC. Therefore, CL3 could be stimulating a non-canonical activation of the inflammasome. Moreover, we wonder whether two key components in inflammasome assembly, NLRP3 and caspase, are required for CL3-induced inflammasome activation in DC. For this, DC from NLRP3 or caspase 1/11 deficient mice (and WT DC as control) were cultured with CL3 or LPS. We observed that IL-1β and IL-18 secretion

by DC from NLRP3 deficient mice, was partially diminished, whereas, in the absence of caspase 1/11, the production of these cytokines was not modified. These results suggest that IL-1β and IL-18 production, promoted by CL3, is partially dependent on NLRP3 and independent on caspase 1/11 activity.

Besides, we evaluated whether CL3 is able to directly cleave pro-IL-1β. In an enzymatic digestion assay, measurable amounts of mature IL-1β were detected when pro-IL-1β was incubated with CL3, and IL-1β levels produced were dependent on CL3 concentration. In contrast, mature IL-1β was not detected when pro-IL-1β was exposed to rvCL3.

Furthermore, we observed that CL3-treated DC were capable to induce IL-13 and IFN-γ secretion in splenocytes in allogeneic cultures ($p<0,05$). This effect was not observed when rvCL3 treated-DC where cultured with allogeneic splenocytes. Interestingly, CL3-treated DC from NLRP3KO mice showed a diminished capacity to stimulate IFN-γ production of allogeneic splenocytes in comparison with CL3-treated DC from Wild type mice.

Our data show that CL3 is endocytosed into DC and is able to cleave pro-IL-1β and pro-IL-18 to their mature and biologically active forms, promoting their release to the extracellular environment, being CL3 cysteine protease activity involved this process. These events are partially dependent on NLRP3 and independent of caspase activity, suggesting that CL3 could exert on DC a caspase like activity undescribed so far. In turn, CL3 treated-DC induce a particular expression of cytokine profile (IFN-γ and IL-13) in allogeneic cultures.

Neutrophil stimulation through CD13 regulates release of neutrophil extracellular traps (NETs)

Pablo Arturo Alvarez, Gloria Erandi Pérez, Enrique Ortega

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
parturo_91@hotmail.com; gera.pfi3@gmail.com; ortsoto@biomedicas.unam.mx

Keywords: Neutrophil Activation, Neutrophils, NETs, CD13, Aminopeptidase N (APN)

Introduction: The immune system is made up of a large number of elements that work together to protect the organism of pathogen invasion. In order to detect pathogens and to mediate effector functions, different types of immune effector cells express on their membrane a vast array of receptors, including receptors that directly recognize pathogen structures, or receptors for opsonins. Both types of receptors are able to initiate signal transduction pathways that mediate its effector functions.

Although CD13 (Aminopeptidase N) is a membrane molecule highly expressed on myeloid cells (monocytes, macrophages, dendritic cells and neutrophils), its possible role in the immune functions of these cells has not been widely explored. A role for CD13 in adhesion processes of monocytes and macrophages has been reported. However, whether CD13 is able to mediate effector functions of neutrophils, has not been explored, except for a possible role of CD13 in apoptosis.

We investigated the role of CD13 as a receptor capable of activating effector functions on PMNs, specifically, to induce the release of neutrophils extracellular traps (NETs). We used two different anti-CD13 Fabs that bind to different epitopes to investigate the effect of CD13 crosslinking on the release of NETs. We found that crosslinking CD13 on the membrane of PMNs modulates NETs release induced by PMA and fMLP and IL-8.

If CD13 (APN) is present in the polymorphonuclear neutrofil (PMN) cells then it is an innate immune receptor capable of mediate effector functions of PMNs like NETs release.

Materials and Methods: Human neutrophils were isolated from peripheral blood of healthy donors using a density gradient. The cells were incubated with Fab fragments of two different anti-CD13 monoclonal antibodies. The cell-bound Fab fragments were crosslinked with secondary F(ab')2 fragments to crosslink CD13 to stimulate and activate the receptor and then activating PMNs with PMA, fMLP and IL-8. We used

Sytox Green to measure DNA release using a Cytation™ 3 Cell Imaging Multi-Mode Reader (BioTek Instruments).

Result: We used Fab-452 and FabC, to induce CD13 cross-linking. Activation of PMNs by CD13 cross linking does not stimulate the cells to release NETs. On the other hand, in cells stimulated by PMA, with CD13 cross linking, there is a reduction of NETs release only when Fab-452 was used in a dose-dependent manner, different of the stimuli fMLP and IL-8.

Conclusions: The crosslinking of CD13 with Fab-452 has a remarkable effect on the release of NETs induced by PMA in neutrophils. Interestingly, although both, the FabC and the Fab452 are specific for CD13, they differently affect NETs release.

Scribble and DLG1 participation during dendritic cell maturation and antigen presentation

Dante Barreda[1], Teresa Santos-Mendoza[2]

[1]Instituto Politécnico Nacional, Mexico City, Mexico
[2]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
leo2_rxi@hotmail.com; tesalonster@gmail.com

Keywords: Antigen Presentation, dendritic cell, siRNAs, Influenza A, NS1

Scribble and Dlg1 belong to the PDZ (postsynaptic density (PSD95), disc large (Dlg), and zonula occludens (ZO-1)) family of proteins because they contain several PDZ domains. These proteins are scaffold proteins highly conserved phylogenetically that have been involved in a wide variety of functions such as protein trafficking, cell signaling and cell polarity in different cell types such as neurons, epithelial cells and lymphocytes. The importance of PDZ proteins in cellular functions is reinforced by the fact that many viruses encode PDZ binding motifs (PDZbm) that mimic endogenous ligands thus interfere with host PDZ-dependent interactions affecting cellular mechanisms that in turn favor viral replication and spread.

Recently, Zheng et al, have described that Scribble is indispensable for the reactive oxygen species (ROS) production in macrophages, thus for bacterial killing by these cells (Zheng, 2016). Nevertheless, the regulation and function of Scribble and Dlg1 is still barely documented in antigen presenting cells (APC). We have recently reported the expression and regulation of Scribble and Dlg1 in human dendritic cells (DC) derived from monocytes, as well as the PDZ-dependent targeting of both proteins by the non-structural protein 1 (NS1) of influenza A (Barreda, 2018). In this work we used siRNAs to knock-down the expression of Scribble and Dlg1 in DC, and performed flow cytometry assays to measure the expression of surface markers, cytokines and co-stimulatory molecules. Furthermore, we analyzed changes in the subcellular localization of both Scribble and Dlg1, during antigen presentation to specific T lymphocytes. Our results demonstrated that Dlg1 is necessary for CD83 up-regulation (Figure 1) and IL-6 production during DC maturation. In addition, we found that Scribble but not Dlg1, re-localized to the IS during antigen presentation. Our results suggest that both proteins Scribble and Dlg1, are involved in relevant functions in the DC biology which is support by our recent findings showing the NS1 PDZ-dependent targeting of both proteins in DC.

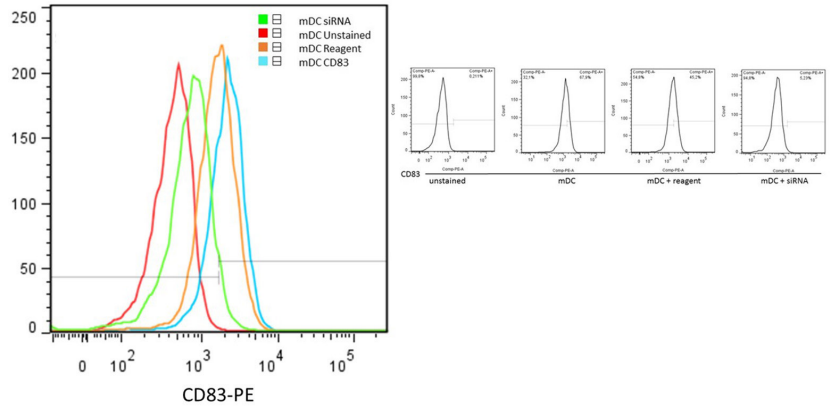


FIGURE 1: DLG1 is required for CD83 upregulation during DC maturation. iDCs were transfected with a mix of siRNA for DLG1 (Dharmacon, Inc) with the reagent Stemfect RNA transfection kit (Stemgent, Inc.) according to the manufacturer's instructions, and the maturation cocktail was added to the culture. 24h latter mDCs were stained with CD83-PE and analyzed by flow cytometry. A) Representative histogram overlay of CD83 expressed on mDC treated (green) or untreated (blue) with DLG1 siRNAs: unstained (red) and only reagent (yellow) controls are included. B) Percentage of CD83+ mDC under each condition.

REFERENCES

Barreda D., Sánchez GM., López FJ., Nava CKE., Bobadilla K Salgado AA, et al. PDZ proteins are expressed and regulated in antigen-presenting cells and are targets of influenza A virus. J Leuko Biol. 2017;1-8.

Zheng W, Umitsu M, Jagan I, Tran CW, Ishiyama N, BeGora M, et al. An nteraction between Scribble and the NADPH oxidase complex controls M1 macrophage polarization and function. Nat Cell Biol. 2016;18:1244–1252.

Mast cells release extracellular traps against isoniazid-resistant Mycobacterium tuberculosis

Marcia Campillo Navarro[1,2], Gloria Mariana Rodríguez López[2], Rodolfo Soria Castro[2], Julieta Luna Herrera[2], Leopoldo Flores Romo[3], Héctor Sumano López[1], Sonia Mayra Pérez Tapia[2,4], Sergio Estrada Parra[2], Iris Estrada García[2], Rommel Chacón Salinas[2,4]

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

[2]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[3]Departamento de Biología Celular (CINVESTAV), México, Mexico

[4]Unidad de Desarrollo e Investigación en Bioprocesos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

marcy265@hotmail.com; Gloria0012@hotmail.com; rodolfo.soria.castro@gmail.com; julietalunah@hotmail.com; lefloresromo@gmail.com; sumano@unam.mx; smpt.2011@hotmail.com; sestradap07@hotmail.com; iestrada5@hotmail.com; rommelchacons@yahoo.com.mx

Keywords: Catalase, Mycobacterium tuberculosis, mast cell, Mast cell extracellular trap, isoniazid-resistant Mycobacterium tuberculosis

Tuberculosis (TB) is a disease of great importance worldwide caused by Mycobacterium tuberculosis (Mtb), which frequently generates drug resistance. Isoniazid has long been used as an effective drug to treat and prevent TB. The resistance of Mtb to isoniazid is usually due to mutations of KatG gene, but loses catalase enzyme activity. Mtb uses this enzyme in combination with peroxide enzymes to protect themselves from the action of reactive oxygen species (ROS) produced by the immune system cells. Mast cells are one of the innate immunity cells that respond to Mtb, internalizing and releasing inflammatory mediators, but it is not known whether Mtb resistance to isoniazide affects Mast Cells Extracellular Traps (MCETs) production, a mechanism that depends on the production of ROS. In the present study we evaluated the formation of MCETs by Mtb starins sensitive and resistant to isoniazid. Mast cells were exposed to PMA, Mtb H37Rv or Mtb isoniazid resistant strain (Mtb KatG-) during different times and fluorescent staining was performed to observe by microscopy or quantify the extracellular DNA. We observed that mast cells released extracellular DNA after 2 hours of stimulation with Mtb KatG- but not against Mtb H37Rv. When we evaluated hydrogen peroxide (H2O2) generation in mast cells stimulated with Mtb strains, we found that Mtb KatG- induced higher levels of H2O2 compared to cells stimulated with Mtb H37Rv. These results indicate that the catalase produced by Mtb decomposes the hydrogen peroxide required for the formation of MCETs.

KIR3DL1, but not its ligands, confers protection for the expression of Acute Lymphoblastic Leukemia (ALL) in Mexican Mestizo patients

Betsy A González-Q[1,2], Miriam Valencia[1], Hilario Flores-A[1,2], Guadalupe Sanchez[2], David Senitzer[3], Clara Gorodezky[1,2]

[1]Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), México, Mexico
[2]Fundación Comparte Vida, A.C., Mexico City, Mexico
[3]City of Hope National Medical Center, Duarte, United States
bgonzalez@fundacioncompartevida.org.mx; mivama@yahoo.com.mx; hilario.flores@salud.gob.mx; rosasenoctubre@yahoo.com; dsenitzer@coh.org; clarag@unam.mx

Keywords: NK cell receptors, KIR genes, Mexican mestizos, 3DL1, 3DS1, ALL-Acute lymphoblastic leukemia

Introduction: Mexico city has one of the highest rates of acute leukemia at 57.6 cases per million and ALL is the most common subtype (85.1%). The disease is clearly polygenic and multifactorial, thus an important role for inherited genetic variation has been suggested for its development. A spectrum of HLA associations were reported and polymorphisms at the killer cell immunoglobulin-like receptors (KIR), influence ALL expression as well. KIR genes expressed on the surface of NK cells and certain T cells, regulate their functions through interactions with their HLA ligands. HLA-C is the dominant KIR ligand, and is documented to be involved in the destruction of leukemic cells as well as other cancers. Due to the important role that KIR-HLA interactions play on the development and activity of NK cells, it is essential to analyze them in the context of association studies. Thus, we addressed this issue by performing a case-control study in Mexican Mestizo patients with ALL, looking for the involvement of KIR3DL1 and its Bw4, A23, A24 and A32 ligands in the expression of ALL.

Methods: All individuals were Mexican Mestizos inhabiting and born in Mexico. The study met the criteria of the declaration of Helsinki. DNA was extracted from peripheral blood with the Maxwell16 instrument from 137 patients (90% were children, 60% were males, age average=17 years) and 356 healthy controls (99.7% were adults, and 52% were males, age average=32 years). KIR3DL1 was detected using a PCR-SSP technique (kits kindly provided by Dr. Senitzer). HLA typing was done using a Luminex PCR-SSOP method. Frequencies of KIR3DL1 and KIR-HLA ligands were compared between patients and controls, using the Chi2 test.

Results: A low frequency of KIR3DL1 in patients vs. controls (80.3% vs. 88.5%; p=0.018; OR=0.530) was observed. While the presence of ligands did not showed differences for Bw4 from HLA-B (p=0.238) or for Bw4 from HLA-A (A23/A24/A32) (p=0.130). The frequency of KIR3DL1 and its ligand Bw4/A23/A24/A32 did not showed differences between patients and controls (p=0.34).

Conclusions: We previously reported associated with protection KIR2DS4 (p=0.002), KIR2DL2 (p=0.001), KIR2DS2 (p=0.0001) and KIR2DS3 (p=0.002) but not KIR3DL1, having typed then, only 66 patients; however, when increasing the number to 137, KIR3DL1 was clearly shown to be a protective gene. We thus conclude that 3DL1 confers a low risk for ALL expression in Mexicans. These data agree with those reported in Chinese, but differ from data found in Canadian Whites and Indians and in admixed Hispanics from California (which include different races); activating KIR2DS1-C2, 2DS2-C1 and 3DS1-Bw4 were risk genes in Indians only. Ethnical differences clearly influence the genetic distribution of KIR genes and their ligands; therefore, probably their associations too. Undoubtedly, KIR3DL1 plays an important role in the expression and clearance of ALL in Mexicans. Moreover, these results are consistent across different ALL phenotypes, as shown in White Canadians. More studies on KIR and its ligands are needed to establish their role on different ethnicities.

Isthmin-1 is expressed in lung by stem-like cells

Carolina Abigail Mendiola, Ricardo Valle Rios, Guadalupe Rivera-Torruco, Hernan Eduardo Herrera-Osorio, Gustavo Alberto Jaimes-Ortega

UNAM, mexico city, Mexico
mendiola.abi@hotmail.com; vallerios@gmail.com; lupitariveratorruco@gmail.com; hernanworks5@gmail.com; gelet321@gmail.com

Keywords: Lung, stem, lineage, HSCs, Isthmin1

Isthmin-1 (ISM1) is a recently described protein, found in the brain isthmus of Xenopus sp., ISM1 contains a TSR domain, an AMOP domain and a signal peptide for extracellular transport. As a soluble ligand it has been reported to interact with integrin αvβ5 on the surface of endothelial cells impairing their capillary network formation; it has also shown that ISM1 induce proapoptotic activity on cancer cells mediated not only by αvβ5 but also through the regulator of UPR response pathway GRP78. Interestingly, ISM1 is also important in patterning craniofacial structures during development.

ISM1 transcripts are found mainly on skin, pericardium, trachea and lung, also its expression has been assessed on CD3low DX5+ lymphocytes; however since ISM1 is expressed in cells derived from the double knock out Rag -/- γc -/- mouse model, probably by lymphocyte precursor cells, we sought to identify which cells are responsible for the ISM1 production using flow cytometry by execution of surface and intracellular labelling. The phenotypical characterization of hematopoietic cells was performed on cells obtained from different tissues but focused on lung, where ISM1 has the most abundant positive population when compared to lamina propria, bone marrow and peripheric blood, reaching around 7.0% of the lymphogate region. When analyzed for CD45, the ISM1+ positive population is found to be roughly 10% positive for this marker. Cell populations other than leukocytes where evaluated; epithelial cells positive for Ep-cam do not show ISM1 expression neither do VEGFR2 positive endothelial cells.

We analyzed specific terminal lineage markers individually, such as CD3, CD4, CD8, CD11b, CD19, CD11c, TER119 and FcRe, and ISM1+ cells where not found related to any of these differentiated blood cell markers. Interestingly, we found a small fraction of cells lacking lineage markers (LIN-) but expressing CD45 and ISM1+, resembling the recently discovered Innate Lymphoid Cells (ILCs) and after including some of the most common ILC markers (CD127, CD161, c-Kit and ST2) only c-Kit is expressed on the surface of LIN-CD45+ISM1+ cells, suggesting that ISM1 is identifying a non-classic ILC population. c-Kit is known to be expressed by undifferentiated cells; accordingly, we

found that the majority of ISM1+ cells are ckit+CD45- cells. Therefore, we performed an analysis including markers commonly known to describe long and short term hematopoietic stem cells (LT-HSCs and ST-HSCs): Sca-1 and CD34. Based on this analysis, we found that ISM1 is expressed by around 50% of LT-HSCs and ST-HSCs cells.

Since stem cells are also considered tissue resident cells, we assessed the presence of β1 integrins associated to residence such as CD49a and CD49b and we were able to identify ISM1+ cells that express Sca1, c-kit and CD34 and CD49a and CD49b.

To investigate if ISM1+ cells may be generated during development, we stained cells from lungs obtained from E16-E18 mice embryos and verified that ISM1+ cells are present; interestingly, we were able to observe that ISM1+ cells are also located in fetal liver.

Thus, our results indicate that ISM1 is a new marker that identifies a population of cells with hematopoietic stem-cell phenotype located in the lung. Whether these stem-like cells expressing ISM1 are actually able to differentiate in lymphoid or myeloid lineages; and therefore playing a role in extramedular hematopoiesis is a subject of current analysis.

Uncaria tomentosa is not cytotoxic neither apoptotic, increases ROS production and induce an M2 polarization in human monocytes-macrophages

Ivan Lozada-Requena[1,2], Bernard Pipy[2], Jose Bernad[2], Melissa Prat[2], Mohamad Alaeddine[2], Cesar Núñez[3], Jose Luis Aguilar[1], Milagros Pinto[1], Sandra Jara[1], Agnès Coste[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru
[2]Université Toulouse III Paul Sabatier, Toulouse, France
[3]Emindes Cáncer, Jesús María, Peru
ivan.lozada@upch.pe; bernard.pipy@inserm.fr; jose.bernad@univ-tlse3.fr; melissa.prat@live.fr; alaeddine_mohamad@live.fr; cesarnunez22@yahoo.com; jose.aguilar@upch.pe; milagros.pinto.d@upch.pe; sandra.jara.a@upch.pe; agnes.coste@univ-tlse3.fr

Keywords: Uncaria, M2, ROS, Monocytes-macrophages, cat´s claw

Uncaria tomentosa is a peruvian liane with immunostimulant properties.

Objective: To determine the effect of a hydroalcoholic extract of Uncaria tomentosa (Cat's claw, 5,03% of pentacyclic oxindole alkaloids, UT-TOA) over cellular viability, production of reactive oxygen species (ROS) and mRNA expression relative of transcripcional markers M1/M2 in human monocytes-macrophages.

Methods: We isolated monocytes-macrophages (1,5x105) from human PBMC allowing their adherence during 1h30min in Macrophage-SFM medium (Invitrogen, CA, USA). Macrophages in culture were washed 2 times with Hanks salts (HBSS+, GibcoTM, France) renewing medium and treating or not with a range of doses of 6,25 – 400 ug/mL UT-TOA to evaluate the cellular viability by MTT, LDH and 7-AAD. For measurement of ROS, macrophages were treated with 25ug/mL of UT for 24h, washed two times in HBSS+, stimulated with 12-O-Tetradecanoylphorbol-13-acetate (TPA, 100ng/well), Zymosan (ZNO, 50ng/well), the chemotactic peptide, N-Formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP, 1uM/well) (SIGMA, France) and Candida albicans (3:1), it was added luminol (60uM) (SIGMA, France) and chemiluminescence emission kinetics was measured for 90min. using a thermostatically (37ºC) controlled EnVisionMulti-label Reader (Perkin Elmer, France). Total RNA from macrophages treated with 25ug/mL for 6h was prepared with RNA Minipreps super kit (Bio Basic, Canada Inc.) using the manufacturer's protocols. The synthesis of cDNA for reverse transcription-quantitative PCR (RT-qPCR) was obtained with Verso cDNA kit (Thermo

Scientific, France). Quantitative real-time PCR was performed on a LightCycler480 system (Roche Diagnostics) using Light Cycler SYBRGreen I Master (Roche Diagnostics). The primers for M1/M2 markers (10mM final concentration) were designed with the software Primer3. Flow cytometry was performed on a FACSCalibur (BD Biosciences) for CD68 (M1) and CD206, CD163 (M2) using specific monoclonal antibodies.

Results: UT-TOA is not cytotoxic neither induce apoptosis in the range of 6,25–100ug/mL. UT-TOA to 25ug/mL significantly increased ROS production by monocytes-macrophages after 24h stimulated with TPA; ZNO; fMLP ($p<0,05$) but non-with C. albicans. UT-TOA to 25ug/mL increased significantly the relative mRNA expression of cd206, cd163, ccr2 after 6h of treatment ($p<0.05$). However, both of them proteins CD206 and CD163 did not change their production after 24h of treatment.

Conclusions: UT-TOA is safe for monocytes-macrophages up to a dose of 100ug/mL. Pretreatment of monocytes-macrophages for 6h with UT-TOA (25ug/mL) induce an M2 polarization to transcriptional level but this polarization is not evident to protein level after 24h. It is possible that there was a relationship between M2 receptors and ROS production but we should demonstrate the increase of these proteins. UT-TOA (25ug/mL) overstimulated ROS production when added drug such as TPA, ZNO and fMLP, which induce endogenous production of superoxide. UT-TOA could enhance innate immune responses against infections or tumors characterized by the presence of antigen glycocidics such as β-glucans.

The hepta-acylated variant of Salmonella Typhimurium lipopolysaccharide causes impaired dimerization of TLR4/MD-2 and reduced pro-inflammatory cytokine production

Gustavo Aldapa-Vega[1,2], Mario Adán Moreno-Eutimio[3], Antonio Berlanga-Taylor[4], Alexis P. Jiménez-Uribe[1], Goreti Nieto-Velázquez[3], Orestes López-Ortega[5], John S Gunn[6], Armando Isibasi[1], Isabel Wong-Baeza[7], Constantino López-Macías[1,8,9], Rodolfo Pastelin-palacios[10]

[1]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Instituto Politécnico Nacional, Mexico City, Mexico
[3]Hospital Juárez de México, Mexico City, Mexico
[4]MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, Faculty of Medicine, Imperial College London, St Mary´s Campus, Norfolk Place, London, United Kingdom
[5]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico
[6]Center for Microbial Interface Biology, Department of Microbial Infection and Immunity, The Ohio State University, Columbus, United States
[7]Laboratorio de Inmunología Molecular II, Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de México, Mexico
[8]University of Oxford, Oxford, United Kingdom
[9]FOCIS Centre of Excellence, Ciudad de México, Mexico
[10]Departamento de Biología, Facultad de Quimica, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
g.aldapa.v@gmail.com; marioadan@inmunoquimica.com; a.berlanga@imperial.ac.uk; athumbix@gmail.com; goretinieto@hotmail.com; letes_01@hotmail.com; gunn.43@osu.edu; isibasi@prodigy.net.mx; isa_wb@yahoo.com.mx; constantino@sminmunologia.mx; rodolfop@unam.mx

Keywords: Salmonella, Toll-Like Receptor 4, adjuvants, endotoxin, lipid A variants

During infection, Salmonella enterica serovar Typhimurium (S. Typhimurium) changes the structure of its lipopolysaccharide (LPS) in response to the host environment. Two LPS lipid A variants have been found, one correspond to LPS with a hepta-acylated lipid A (LPS 430) and the other to a LPS with modified phosphate groups on its lipid A (LPS 435). We have previously shown that these structurally modified LPS could participate in the subversion of immunity to S. Typhimurium infection by reducing both innate and adaptive immune responses to the bacteria in mice. Nevertheless, it is not know if LPS 430 and 435 could also subvert the innate immune responses in human cells. In the present study we found that LPS 430 and LPS 435 are less

efficient than LPS WT to induce the production of pro-inflammatory cytokines by human monocytes, LPS 430 induced similar nuclear translocation of the transcription factors NF-κB and IRF3, compared to LPS WT and LPS 435. Microarray analysis of LPS 430- and 435-activated monocytes revealed that inflammatory gene transcription profile similar to the transcription profile induced by LPS WT but showed different expression of miRNAs and small nucleolar RNA. Moreover, the response to LPS 430 was associated with decreased dimerization of the TLR4/MD-2 complex suggesting that structurally modified LPS are sensed differently than WT by this receptor. Taken together these results suggest that differences in the cytokine profile induced by 430 and 435 LPS in human monocytes is due to a differential recognition by TLR4/MD2 that resulted in different transcription profile of several sets of genes. These results are relevant to understand LPS modulation of immune responses and immunity and could be relevant to develop novel adjuvants and immunomodulators.

Distribution pattern of the natural cytotoxic receptor NKp30 in NK subpopulations following the natural history of the cervical cancer

Gloria Yareli Gutierrez Silerio[1], Miriam Ruth Bueno[1], Marta Escarra Senmarti[1], Alejandra Natali Vega[1], Oscar Joel Carrillo Garibaldi[1], Jorge Gutiérrez Franco[1], Martha Eloisa Ramos Márquez[1], Fabiola Solorzano Ibarra[1], Jesse Haramati[3], Susana del Toro Arreola[1]

[1]University of Guadalajara, Guadalajara, Mexico
[2]Hospital Civil de Guadalajara, Guadalajara, Mexico
[3]Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, Mexico
gloriagutierrezsilerio93@gmail.com; ruthmyriamtop@hotmail.com; escarra.marta@gmail.com; natalivega.dcb@gmail.com; drojcarrillog@hotmail.com; qfb_jorgeguf@hotmail.com; eloisa10@yahoo.com; fabinami@hotmail.com; jharamati@gmail.com; susana@cucs.udg.mx

Keywords: cervical cancer, Natural Citotoxicity Receptors, NKp30, NKp30 isoforms, B7H6

Background: Due to the link between genital human papillomavirus infection and cervical cancer development, it is unquestionable that the immune system has a main role in the manteinance or in the clearance of the lesion. At this point, it is well known that NK cells are crucial in the elimination of virus-infected and tumor cells through a variety of different activating receptors, such as NKp30. NKp30 is a transmembrane-I glycoprotein with the ability to associate with signaling molecules leading to the activation of the NK cell cytotoxic machinary. NKp30 has also generated interest due to the presence some isoforms generated by alternative splicing. At the moment, three isoforms named NKp30a, NKp30b and NKp30c have been characterized. The first two isoforms promote the exocytosis of the cytotoxic granules, while NKp30c is rather an immunoregulatory isoform. However, at the moment it is not known the distribution pattern of the different NKp30 isoforms during the natural history of the cervical cancer.

Objective: To reveal the expression pattern of the different NKp30 isoforms in human peripheral NK cells following the natural history in the development of cervical cancer.

Methods: Patients with invasive cervical carcinoma and precursor lesions, as well as healthy women were enrolled in the study. Then, peripheral blood with or without anticoagulant was collected to obtain mononuclear cells or sera. In order to characterize the molecular NKp30 isoforms, RT-qPCR based analysis was used. The total

mRNA extraction was carried out using the TRIzol® technique and each isoform was determined using specific probes and primers, normalizing with β2-microglobulin gene. The Corbett thermal cycler © was used for the analysis. In order to go deeper in the characterization of the NKp30 receptor, a multicolor flow cytometry staining protocol was used to analyze the expression of this receptor in subclasses of NK cells: CD56dullCD16bright, and CD56brightCD16dull. The BD FACSCanto II® flow cytometer was used for the Reading and the FlowJo® software was used to analyze the data. Additionally, ELISA assays were used in order to quantify soluble B7-H6 (an endogenous ligand for NKp30).

Results: Regarding the expression of NKp30 mRNA, we observed a predominance of the isoform B compared with the isoforms A and C, in the control group, which is in accordance with some previous reports. At the moment, we do not know yet if this predominance is also observed in the cancer or lesions groups (experiments under process). With respect to cytometry and ELISA, these experiments are also still under performance.

Determination of metastasic biomarkers expression levels in circulating tumor cells in Diffuse Large B Cell Lymphoma patients

Rafael Ceron Maldonado[1], Adolfo Martínez Tovar[1], Christian Omar Ramos[1], Maria Isabel Soto Cruz[2], Laila Gutiérrez Kobeh[3], Etta Rozen Fuller[1], Iveth Mendoza Salas[1], Anel Irais García Laguna[1], Adrián De La Cruz Rosas[1], Juan Collazo Jaloma[1], Irma Olarte Carrillo[1]

[1]Hospital General de México, Mexico City, Mexico
[2]Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, Mexico City, Mexico
[3]Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
cmrafael.bh@gmail.com; mtadolfo73@hotmail.com; leukemiachop@hotmail.com; sotocruz@unam.mx; lgutierr@unam.mx; etta.rozen@gmail.com; iveth_ms@hotmail.com; gala.anel@gmail.com; adrian.dlcr@outlook.com; jcollazo@hotmail.com; irmaolartec@yahoo.com

Keywords: Lymphoma, metastasis, circulating tumor cells, RT-qPCR, overexpression

Introduction: Diffuse Large B Cell Lymphoma (DLBCL) is the most frequent onco-hematologic neoplasm in Mexico. Around 60% of DLBCL cases are diagnosed on stage III or IV, when cancer cells have migrated from the primary tumor to other anatomic sites (metastasis), which constitutes the worst prognostic criteria in DLBCL and cancer patients.

The aberrant expression of genes implied in proliferation and cell-dead resistance, such as BCL2, BCL6 and Myc, have been studied in DLBCL in lymph node; however, those involved in replicative immortality, angiogenesis and extracellular matrix degradation on primary tumor, that result in the release of Circulating Tumor Cells (CTC) from the primary tumor to distant sites in the organism, has not been addressed and could be potentially integrated to those already mentioned. In several types of cancer, like breast cancer, lung cancer, liver cancer, ovary cancer and melanoma, it has been determined an association between CTC presence and progression of the malignancy, early relapse and low overall survival. Particularly in DLBCL, CTC dissemination is a common phenomenon, therefore, more than half of these cases are diagnosed when more than one anatomic site has been infiltrated with cancer cells. Due to the few works of CTC in DLBCL, the need of this study arises.

Objective: To determine the expression levels of metastatic biomarkers (BLC6, BCL10, CK19, KI67, TWIST1, hTERT, VEGFR1, VEGFR2, MAGE-A3), that indicate the presence of CTC in peripheral blood of DLBCL patients at diagnosis and at treatment follow-up. To make a co-relation analysis between the metastatic biomarkers expression and pathological-clinical parameters of the patients.

Methods and Materials: Longitudinal and analytical study that included 30 DLBCL patients diagnosed by hematology and pathology services of Hospital General de México. Dr. Eduardo Liceaga, prior informed consent. The clinical follow comprehend the peripheral blood extraction at 3 different times: at diagnosis (T0), in the middle of the treatment (T1) and at the end (T2) of the R-CHOP treatment (Rituximab, Cyclophosphamide, Vincristine and Prednisone; a cycle every 21 days). Mononuclear cells were isolated by density gradient (Ficoll Hypaque™) for the total RNA extraction with TRIzol™ (Invitrogen, Life Technologies Carlsbad, CA). cDNA were synthetized using 2000 ng of RNA and Oligo dT and MMLV (PROMEGA, Madison WI, USA). The quantitation of the genes was made through RT-qPCR on Step One™ thermocycler (Applied Biosystems, Life Technologies), using 2-ΔΔCт method to determine the relative expression levels of each one of the biomarkers. Hydrolysis probes BCL6 (Hs00153368_m1), BCL10 (Hs00961847_m1), KI67 (Hs01032439_m1), CK19 (Hs00761767_s1), VEGFR1 (Hs010562961_m1), VEGFR2 (Hs00911700_m1) and MAGE-A3 (Hs00366532_m1), and β-2-microglobulin (Hs00985689_m1) as reference gene, were used to detect the biomarkers. 60 samples of healthy donors were used as negative controls.

On the other hand, the patients were followed up at a time of 126 days, collecting clinical data which involved age, gender, clinical stage, extranodal sites, ECOG, DHL, white-blood count, histological origin, response by PET at the middle and the end of treatment, refractoriness, relapses and death.

The Anderson-Darling normality test, t-test and Chi-square test were performed on IBM SPSS 23 software (Statical Package for Social Sciences, SPSS Inc., Chicago, USA).

Results: The over-expression frequencies of patients at three times (T0, T1 and T2) are showed in Table 1.

Table 1. Expression frequency of metastatic biomarkers in patients with DLBCL at T0, T1 and T2

Biomarker	Patients T0 (%)	Patients T1 (%)	Patients T2 (%)
KI67	14 (47)	7 (23)	4 (13)
BCL6	10 (33)	3 (10)	1 (3)
BCL10	9 (30)	4 (13)	2 (7)
VEGFR1	8 (27)	2 (7)	2 (7)
TWIST1	7 (23)	1 (3)	1 (3)
CK19	6 (20)	6 (20)	2 (7)
hTERT	2 (7)	0	0
MAGE-A3	1 (3)	0	1 (3)

It was determined that at T0, 87% (26/30) of patients had overexpression levels of at least one biomarker, being Ki67 and BCL6 the genes with the higher over-expression frequency (47% and 13% respectively). At T1, 43% (13/30), and at T2, 33% (10/30) of patients over-expressed at least one metastatic biomarker, wich suggests the prevalence of tumor processes in these patients.

Significant differences between healthy donors and patients were observed ($p < 0.05$) through the Anderson Darling and t- tests, which validates the quantitative difference between the expression levels detected by this method.

The clinical importance of the detection of the biomarker panel was observed through a statistically significant correlation after the Chi-square test ($p < 0.05$) between the refractoriness to the treatment and the expression of MAGE-A3, which has been described in the literature. There was also an association between CK19 and high IPI values. In addition, a correlation was found between the over-expression of BCL6 and refractoriness to treatment, as well as the expression of TWIST1 and relapses. The association with expression of biomarkers at T1 and T2 demonstrates the importance of molecular monitoring in cancer patients.

Conclusion: The presence of CTC was determined by the expression levels of metastatic biomarkers (BCL6, BCL10, CK19, KI67, TWIST1, hTERT, VEGFR1 and MAGE-A3) in patients with LDCGB both at diagnosis and follow-up, which may constitute an aspect to be considered as part of the evaluation and modification of the costly treatment scheme, including clinical monitoring and the potential prediction of early relapses.

REFERENCES

Alix-Panabières C, Pantel K. (2013). Circulating tumor cells: liquid biopsy of cancer Clin Chem; 59(1):110-8.

Hong, B., Zu, Y. (2013). Detecting Circulating Tumor Cells: Current Challenges and New Trends. Theranostics, 3(6), 377–394.

Wallwiener M, Hartkopf AD, Baccelli I, et al. (2013). The prognostic impact of circulating tumor cells in subtypes of metastatic breast cancer. Breast Cancer Res Treat. 2013;137(2):503-10

Bayarri C., Ortega. G., Cueto A., et al. (2016). Circulating Tumor Cells Identify Early Recurrence in Patients with Non-Small Cell Lung Cancer Undergoing Radical Resection. PLoS ONE, 11(2), e0148659

Lu Y, Wang P, Wang X, et al. (2016). The significant prognostic value of circulating tumor cells in triple-negative breast cancer: a meta-analysis. Oncotarget, 7(24), 37361–37369.

Paluchowski B, Krawczyk N, Fehm T. (2016). Potential Role of Circulating Tumor Cell Detection and Monitoring in Breast Cancer: A Review of Current Evidence. Frontiers in Oncology, 6, 255.

Lenz G, Staudt L. (2010). Aggressive lymphomas. N Engl J Med; 362(15):1417-29

Cervical cancer cells are induced to produce TGF-β through CD73-adenosine pathway: a preliminar report

Rosario García-Rocha[1], Alberto Monroy García[2], Luis Roberto Avila Ibarra[2], Monserrat Carrera-Martínez[2], Vianey Gutiérrez-Serrano[2], María del Carmen Fuentes-Castañeda[2], Jorge Hernández-Montes[1], Benny Weiss-Steider[1], María Lourdes Mora-García[1]

[1]Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, Mexico City, Mexico

[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico

rgrbiologia@hotmail.com; inmunologiaycancer@hotmail.com; luischaper@hotmail.com; monsecarreramtz@gmail.com; vianydany@hotmail.com; carminamia@hotmail.com; jhzms@yahoo.com; bennyweiss@hotmail.com; lulumora@yahoo.com

Keywords: Adenosine, cervical cancer, TGF-beta, adenosinergic pathways, CD73 expression

Introduction: Cervical cancer (CeCa) is a major public health problem in our country, each year about 10,000 new cases of invasive CeCa are diagnosed [1]. It has been reported that high immunohistochemical expression of TGF-β as well as high levels of this cytokine in plasma, predicts a poor prognosis in CeCa patients [2]. However, until now, the mechanisms that induce the production of this cytokine have not been completely dilucidated. Recent reports suggest that extracelular cell signaling by adenosine (Ado) in tumor tissues could mediate TGF-β production, since acumulated Ado into tumor microenvironment is also associated with high levels of TGF-β [3–5]. Ado is generated by the functional activity of the ectonucleotidases CD39 and CD73 which hydrolyse ATP and ADP to AMP, and AMP to Ado, respectively. Most extracellular signaling activities conducted by Ado are mediated by adenosine receptors (ARs: A1, A2A, A2B, and A3) coupled to G proteins and are located on the membranes of target cells. [6]. In fact, it has been reported that Ado signaling in T lymphocytes through A2A receptor can induce the production of TGF-β [7]. On the other hand, we have recently reported that CeCa derived cell lines express high levels of CD73 and are capable to produce high amounts of Ado when are cultured in the presence of AMP [8].

Objective: To analyze the participation of the CD73-adenosine pathway in the generation of TGF-β in CeCa tumor cells.

Materials and Methods: MS751 (HPV-18, CD73 +), CaSki (HPV-16, CD73 +) and C33A (HPV-, CD73-) CeCa cell lines were used. To analyze the participation of CD73 in the generation of Ado by AMP hydrolysis, CD73 was downreguated in MS751 and CaSki cells by using double-stranded RNA interference molecules inserted in a pSIREN vector (pSiren-CD73 siRNA), pSiren-luciferase vector was used as a transfection control. Wild type as well as CD73-downregulated CeCa cell lines were cultured during 96 h in the presence of 1mM of either AMP or Ado. The expression of CD73 in CeCa cells was performed by Flow cytometry using anti-CD73-PE monoclonal antibody, and by qRT-PCR using specific primers to CD73 and G6PDH gene which was used as a constitutive control. The catalytic activity of CD73 was carried out by incubation of CeCa cells in the presence of AMP. After 96 h, the generation of adenosine was evidenced by thin layer chromatography. TGF-β1 was determined by ELISA using a (TGF-β1 Quantikine ELISA Human Kit).

Results: Constitutive expression of CD73 on MS751 and CaSki cell lines was performed by flow cytometry. In order to analyse the functional activity of this ectonucleotidase, we previously inhibited its expression by transfection of the cell lines with a specific pSiren-CD73 siRNA. More than 70% of CD73 expression was diminished in MS751 and CaSki cell lines (Fig. 1B-C). C33 cell line was used as a negative control for CD73 expression (Fig. 1A). Significant decrease in the relative expression of mRNA for CD73 was also observed in the pSiren-CD73 siRNA transfected cells in comparison with that transfected with a pSiren-luciferase vector (Fig. 2). The activity of CD73 to hydrolyze AMP to Ado was detected in supernatants of MS751 and CaSki cells as well as in cells transfected with pSiren-luciferase vector. However, Ado was almost not detected in supernatants of cells transfected with pSiren-CD73 siRNA (Fig. 3). To determine the participation of the CD73-adenosine pathway in the secretion of TGF-β, the presence of this cytokine was quantified in supernatants of CeCa cells cultured in the presence of AMP or Ado by ELISA. We observed that supernatants of CaSki (Fig. 4A) and MS751 (Fig. 4B) cells as well as that of cells transfected with pSiren-luciferase vector, showed a significative higher amounts of TGF-β relative to their respective basal production. Interestingly, we did not observed increase of TGF-β in supernatants of CaSki and MS751 cells transfected with pSiren-CD73 siRNA (Fig.4A and B).

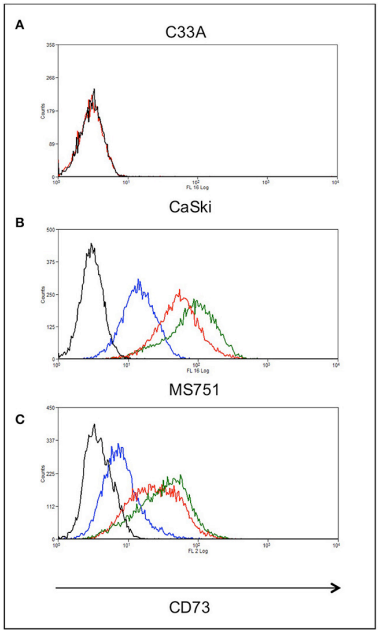


FIGURE 1: Decrease of CD73 expression in CaCU cell lines after being transfected with pSiren-CD73 siRNA. The expression of CD73 was analyzed in a basic manner in the cell lines and flow cytometry was stained with anti-CD73-PE antibody, C33A (A), CaSki (B) and MS751 (C).♦ autofluorescence, ♦ basal expression, ♦ pSiren-luciferase vector, ♦ pSiren-CD73 siRNA.

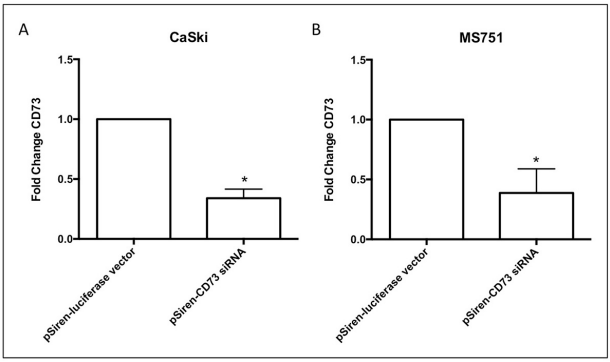


FIGURE 2: Decrease in mRNA expression for CD73 in CaCU cell lines after being transfected with pSiren-CD73 siRNA. The expression of CD 73 mRNA was analyzed in both CaSki (A) and MS751 (B) cells. The results are represented as the mean ± SD of three experiments. * Indicates a significant difference with respect to its baseline control ($p < 0.05$).

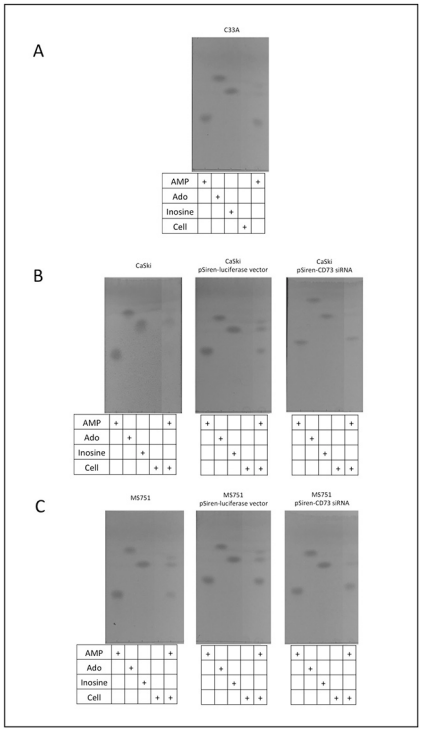


FIGURE 3: Functional activity of CD73 in the different CaCu cell lines. The enzymatic activity of CD73 was analyzed by TLC in C33A (A) CaSki (B) and MS751 (C) cells at 96 h of culture in the presence of AMP or Ado.

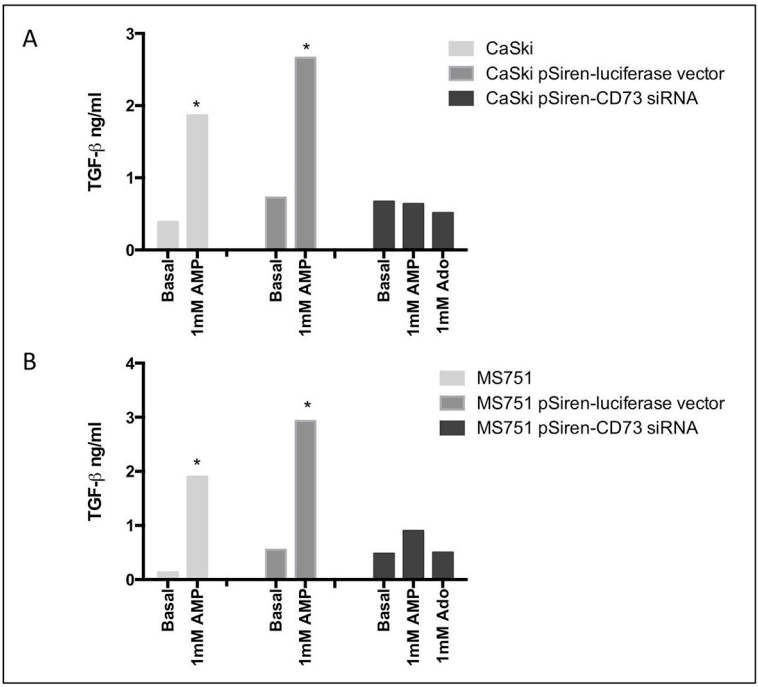


FIGURE 4: Quantification of TGF-β. ELISA assays were performed to determine the TGF-β content in supernatants of CaSki (A) and MS751 (B) on parental cell, pSiren-luciferase vector cells and pSiren-CD73 siRNA cells, the supernatants was analyzed at 96 h of culture in the presence of 1mM of AMP or Ado. * Indicates a significant difference with respect to its basal control ($p < 0.05$).

Conclusions: These results suggests that adenosine produced by hydrolitic activity of CD73 in CeCa cells can strongly contribute in the generation of TGF-β.

REFERENCES

[1] INEGI. Estadísticas a propósito del día mundial contra el cáncer (4 de febrero). Datos nacionales. Aguascalentes, Ags. México2016.

[2] Diaz-Benitez CE, Navarro-Fuentes KR, Flores-Sosa JA, Juarez-Diaz J, Uribe-Salas FJ, Roman-Basaure E, et al. CD3zeta expression and T cell proliferation are inhibited by TGF-beta1 and IL-10 in cervical cancer patients. J Clin Immunol. 2009;29:532-544.

[3] Beavis PA, Divisekera U, Paget C, Chow MT, John LB, Devaud C, et al. Blockade of A2A receptors potently suppresses the metastasis of CD73+ tumors. Proc Natl Acad Sci U S A. 2013;110:14711-14716.

[4] Resta R, Yamashita Y and Thompson LF. Ecto-enzyme and signaling functions of lymphocyte CD73. Immunol Rev. 1998;161:95-109.

[5] Regateiro FS, Howie D, Nolan KF, Agorogiannis EI, Greaves DR, Cobbold SP, et al. Generation of anti-inflammatory adenosine by leukocytes is regulated by TGF-beta. Eur J Immunol. 2011;41:2955-2965.

[6] Fredholm BB, AP IJ, Jacobson KA, Linden J and Muller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update. Pharmacol Rev. 2011;63:1-34.

[7] Stagg J, Divisekera U, McLaughlin N, Sharkey J, Pommey S, Denoyer D, et al. Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. Proc Natl Acad Sci U S A. 2010;107:1547-1552.

[8] Ávila-Ibarra LR. Análisis funcional de la vía adenosinérgica (CD39/CD73/adenosina) como mecanismo inmunosupresor de células tumorales de cáncer de cuello uterino. México: UNAM; 2014.

STAT6 attenuates inflammation and drives protection against colitis-associated colon cancer

Yael Gabriela Delgado-Ramirez, Yadira Ledesma-Soto, Luis Ignacio Terrazas, Sonia Andrea Leon-Cabrera

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
yael_dery@hotmail.com; lesy_790413@yahoo.com; literrazas@unam.mx; soleon81@gmail.com

Keywords: Cell Biology, colorectal cancer, tumor, STAT6, immunology, Tregs, CAC, AOM/DSS, K.O Mice

Colitis-associated colon cancer (CAC) is the third leading cause of death from cancer worldwide.(1) The signal transducer and activator of transcription (STAT6) plays an important role in proliferation and metastasis, as well as in the modulation of intestinal homeostasis, immune response and cell signaling (2). In our working group, using an experimental model of CAC that shows similarities to the sporadic cancer that develops in humans, we determined that 70% of STAT6 deficient animals (STAT6-/-) did not develop tumors and displayed a decrease in leukocytic infiltrate and in pro-inflammatory cytokine production in the colon compared withwild-type (WT) animals, suggesting that immunomodulatory mechanisms may be involved (3). Several experimental models indicate that STAT6 can negatively regulate the activity of regulatory T cells (Treg) (4), however, their relationship during CAC is unknown. To investigate if the decrease in tumor development in the absence of STAT6 is due to alterations in the recruitment of Treg cells that could modulate the intestinal immune response during the early stages of CAC, AOM / DSS was administered to WT mice and STAT6-/- mice. Body weight changes,the rate of disease damage and tumor development was evaluated during the experiment. The animals were sacrificed on days 20, 40 and 68 where changes incytokine expression and in the number of CD4 + CD25hi Foxp3+ cells were determined in mesenteric lymph nodes, spleen and circulation. It was determined that the deficiency of STAT6 prevents the development of CAC. The decrease in tumorigenesis was associated with a lower inflammatory infiltrate, a decrease in the expression of proinflammatory cytokines and high levels in the expression of anti-inflammatory cytokines IL-10 and TGF-β in STAT6-/- mice. It was determined that CD4 + CD25hiFoxp3 + cells increased significantly in STAT6-/- animals during early stages of tumor development (day 20) compared to WT animals in circulation and NLM. This increase gradually decreased throughout the treatment and induction of CAC. Also the expression level of the Foxp3 intracellular protein associated with the Tregs function was increased in the colon. These results sugest that The absence of STAT6 alters the recruitment of Tregs cells. Therefore, it will be important to charac-

terize markers associated with the activation of Treg cell populations and to evaluate whether they present differences in their suppressive activity in dependence on STAT6.

REFERENCES

1. Jemal, A., et al., (2004) Cancer statistics, 2004. CA: a cancer journal for clinicians. 54 (1):p 8-29
2. Li, B. H., X. Z. Yang, et al. (2008). "IL-4/Stat6 activities correlate with apoptosis and metastasis in colon cancer cells." Biochem Biophys Res Commun 369(2): 554-560.
3. Leon-Cabrera, S.A., et al. (2017). "Lack of STAT6 attenuates inflammation and drives protection against early steps of colitis-associated colon cancer". Cancer Immunol Res,. 5(5).
4. Takaki Hiromi, Ichiyama Kenji, Keiko Koga, Takatoshi Chinen, Giichi Takaesu, Yuki Sugiyama, Shigeaki Kato,Akihiko Yoshimuray Kobayashi Takashi. (2008) STAT6 Inhibits TGF-1-mediated Foxp3 Induction through Direct Binding to the Foxp3 Promoter, Which Is Reverted by Retinoic Acid Receptor*. J Biol Chem. 283 (22):14.955- 14.962

Prophylactic antitumoral adjuvant effect of Cry1Ac protoxin and toxin on breast cancer murine model

Roberto Raul Servin Garrido, Leticia Moreno-Fierros, Damaris Ilhuicatzi Alvarado

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
robertorserving@hotmail.com; lemofi@unam.mx; ilhuicatzidamaris@hotmail.com

Keywords: Adjuvants, Immunologic, Immunotherapy, Vaccines, Cancer, Cry1Ac proteins

Breast cancer is the most common cancer type in woman worldwide. Breast cancer patient treatment consists of surgery followed by chemotherapy, radiotherapy or hormonal therapy. Despite the existing therapies, it is common interest to develop, new therapeutic and prophylactic strategies, capable of specifically eliminating tumor cells. Nowadays, immunotherapy has emerged as an attractive therapy for the treatment for several types of cancer.

Immunotherapy is based upon the stimulation of the immune system, looking forward to detect and eliminate tumor cells. Due to the specificity of the antitumor immune response and immunological memory, the field of immunotherapy has grown exponentially in recent years. One strategy that has been used in immunotherapy against cancer, is the use of adjuvants, which are based on activating innate immunity and improve the antigen tumor presentation; they have been either administrated alone, or combined with tumor lysates or tumor peptides. Some types of adjuvants, such as TLR agonists, have been studied, however, some studies have shown a wide variety of tumor types expressing TLR, favoring resistance to apoptosis, metastasis and proliferation. Therefore, it is important to find new adjuvants that are safe in their application and do not favor the growth of tumors.

Cry1Ac protoxin and toxin are two forms of a protein produced by Bacillus thuringiensis. Cry1Ac protoxin is a potent immunogen and systemic and mucosal adjuvant. It has protective adjuvant effect against three models of parasitic infections. The mechanism by which the Cry1Ac protoxin has adjuvant effects is due to the ability to activate macrophages inducing upregulation of costimulatory molecules (CD80 and CD86) and inducing the production of pro-inflammatory cytokines (IL-6, INF-γ, TNF- α and MCP-1). Cry1Ac toxin also has the ability to activate macrophages by inducing upregulation of costimulatory molecules (CD80, CD86 and ICOS-L) and inducing the production of pro-inflammatory cytokines (TNF-α and IL-6). The protective adjuvant effect of Cry1Ac protoxin and toxin

could be extended to other non-infectious diseases such as cancer. The Cry1Ac protoxin and toxin could have the ability to activate innate immunity cells and induce an anti-tumor response.

To evaluate the antitumor effect adjuvant of Cry1Ac protoxin and toxin, we used a murine model of breast cancer tumor cells line 4T1, which simulates phase IV breast cancer in humans. Two different immunization schemes were used, the first one, a short scheme, with 3 prophylactic immunizations every 3rd day; and the second, a long scheme, to improve the adaptive immune response, which consisted of 3 prophylactic immunizations every 7th day. BALB/c mice were immunized with lysate of the cell line 4T1 + Cry1Ac protoxin or toxin intraperitoneally. To establish subcutaneous tumors, cells viable 4T1 cells were injected into left mammary fat pad of BALB/c mice. The tumor growth was monitored every 3rd day. The mice were sacrificed 40 days after the inoculation of the tumor cells.

Short immunization scheme, the mice immunized with 4T1 cell lysate + Cry1Ac protoxin or toxin show antitumor adjuvant effect, because they showed a lower tumor growth to the control groups (cell lysate 4T1 alone and PBS) and a higher percentage of tumor-free mice. We evaluated changes in CD3+, CD4+ and CD8+ lymphocyte populations in splenocytes of the mice that received the different treatments. The mice that received the Cry1Ac protoxin or toxin as adjuvants maintained the number of CD3+, CD4+ and CD8+ lymphocytes as well as the healthy mice, while the mice that developed tumor growth (cell lysate 4T1 alone and PBS), had a decrease in the amount of CD4+ and CD8+ lymphocytes, they have an important role in the elimination of tumor cells. Subsequently, we evaluated the in vitro proliferation of CD4+, CD8+ and CD19+ lymphocytes, stimulated with lysate 4T1 in spleen cells of the mice that received the different treatments. Despite, at this time point analyzed (40 days), we did not observe differences in the percentage of proliferation of lymphocytes between the different treatments, it is important to note the groups coadministered with Cry1Ac protoxin or toxin presented higher amount of proliferating T cells as they maintained the proportions of T lymphocytes while the mice with administered just with the vehicle or immunized with lysates alone presented a significant reduction in the amount of T lymphocytes.

In the long immunization scheme, interestingly, mice immunized with 4T1 cell lysate + Cry1Ac protoxin were protected and did not develop tumors (Figure 1). The protected mice maintain the amount of CD3 +, CD4 + and CD8 + lymphocytes in the same way as healthy mice, while those that developed tumor (cell lysate 4T1 alone and PBS) show a decrease in the amount of these lymphocytes. We evaluated the proportion of myeloid derived suppressor cells (MDSC) which have been reported in cancer patients and mouse models that are increased, MDSC suppressing the antitumor response of

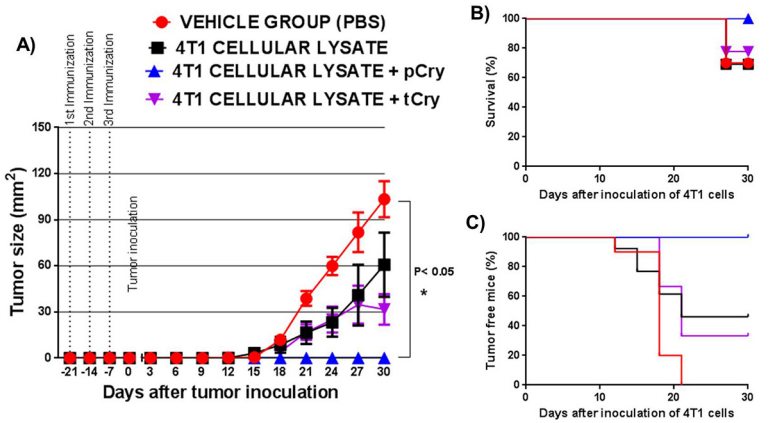


FIGURE 1

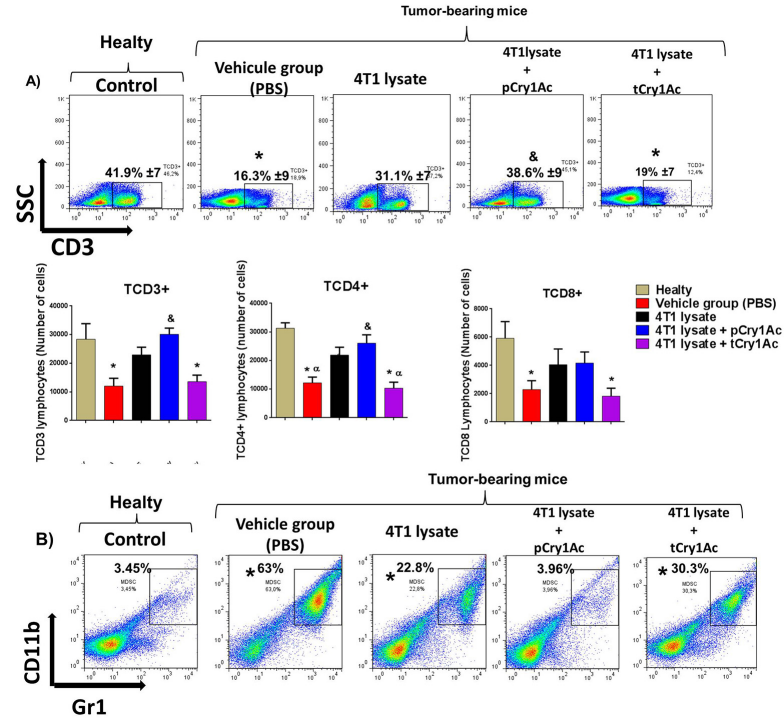


FIGURE 2

effector lymphocytes and promote tumor growth. Mice immunized with 4T1 cell lysate + Cry1Ac protoxin showed a minor amount of MDSC similar to that found in healthy mice, while those that developed tumors have a marked increase in the proportion and amount of MDSC (Figure 2). A possible mechanism by which mice that received the Cry1Ac protoxin as an adjuvant is completely protected from the development of tumors, which could prevent the establishment of the tumor, avoiding the presence of MDSC which contribute to favor the immunosuppressive environment and tumor growth. In contrast to the augmented protective effect conferred by coadministration with Cry1Ac protoxin using the long immunization scheme immunization with cell lysate 4T1 + Cry1Ac toxin showed no improvement with respect to the short immunization scheme. But the protective antitumoral effect was still higher than the one conferred by immunization with cell lysate 4T1 alone.

We continue characterizing the immunological mechanism by which Cry1Ac protoxin confers significant antitumoral adjuvant effect, especially with the long immunization scheme. The outcomes sustain that Cry1Ac protoxin is a promising adjuvant strategy to achieve prophylactic antitumoral immunity, as in this study using the mouse model of breast cancer the emergence of tumors was prevented by immunization of Cry1Ac protoxin with 4T1 cell lysate.

Cytotoxic effect of the ethanolic extracts of fresh and dry leaves of Annona muricata L. in breast cancer cell lines

María del Rosario Huizar-López, Itzel Citlalli Alcázar-Ríos, Patricia Castro-Félix, Josefina Casas-Solis, Anne Santerre

Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, Mexico

mhuizarl@yahoo.com.mx; itzel_alcazar@hotmail.com; patriciacastrofelix@gmail.com; jcasasmicro@gmail.com; asanterre@live.com.mx

Keywords: Breast, Cancer, Cytotoxicity, polyphenols and health, Annona muricata leaves

Introduction: Worldwide, cancer is one of the main causes of death, and in 2015 cancer was responsible for approximately 8.8 million deaths (OMS, 2017). Breast cancer is a multifactorial disease and one of the five principal types of cancer, and is the second leading cause of cancer-related death among women (Najmuddin et al., 2016). Carcinogenesis involves the accumulation of genetic mutations over a long period of time as well as epigenetic alterations that affect, proto-oncogenes, tumor suppressor genes and DNA repair genes, leading to clonal expansion and unbalance between cell death and cell proliferation, conferring cells advantages for division and transformation (Minari and Okeke, 2014). Early detection of breast cancer plays an important role in the fate of the patient. The most common symptoms of this disease are painless small masses within the breast; detection through palpation and inspection is very useful to detect them. The examination of the breast tissue by means of mammography is further helpful to detect abnormal structures indicative of some type of carcinogenesis. Finally a biopsy may be needed, as well as surgery to eliminate potentially malignant tumors (Vainio and Bianchini, 2002). Breast cancer treatment typically involves surgery with the partial or total removal of the breast, radiotherapy and chemotherapy. The precise treatment strategy depends on the age of the patient and the stage of the cancer at the time of diagnostic. In most cases a combination of treatments leads to better outcomes (Angulo et al., 2013).

Traditional home remedies of natural origin have been used empirically, but successfully, for the prevention or treatment of diverse diseases. This has led to the study of their possible mechanisms of action and the characterization of the principal groups of molecules responsible for their biological activities. It has been shown that plants rich in polyphenols present strong antioxidant and antitumoral activities, amongst other interesting properties. These studies have led to the development of several innovative

alternatives, some of which already contribute to cancer prevention or treatment. One of the advantages of extracts of natural origin is their ability to discriminate between normal and cancer cells. This makes them less invasive and more specific than surgery, chemo and radiotherapy, with minor negative side effects (Muñoz and Ramos, 2007).

Annona muricata L. commonly known as Guanabana or Graviola in Latin America, is extensively used as a traditional medicinal plant against an array of human diseases, especially cancer, parasitic infections, hypertension and diabetes (Kim et al., 2016; Najmuddin et al., 2016). Guanabana grows under warm and humid climates and can be found in tropical deciduous forest areas, especially in the tropical zone of Central and South America, East of Africa and South East of Asia. In Mexico it is present in the occidental and south parts of the country, in the states of Chiapas, Guerrero, Jalisco, Quintana Roo, Tabasco, Veracruz and Yucatan (Coria et al., 2016). The leaves of A. muricata are known to be rich in polyphenols and have been studied for their cytotoxic activity in several cancer types, in vivo and in vitro to determine the mechanism of this plant against cancer cells proliferation.

Aim of the study: To study the cytotoxic effect of ethanolic extracts from fresh leaves (EFLAM) and dry leaves (EDLAM) of Annona muricata in the breast cancer cell lines (MC-F7 and HCC1954), in immortalized keratinocytes (Hacat), and in peripheral blood mononuclear cells (PBMC).

Materials and Methods: The leaves of Guanabana were harvested from Tepic, Nayarit, Mexico and identified as Annona Muricata L. in the Instituto de Botánica (IBUG) from the Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA), University of Guadalajara; Mexico. Ethanolic extracts were obtained from fresh and dry leaves, concentrated by using a rotary evaporator, lyophilized and stored for further analysis. Polyphenol concentration of the different extracts was determined using the Folin method. The cytotoxic activity of decreasing concentrations of the leaf extracts (1000, 500, 250 and 125 μg/mL for 48 h) was evaluated in two breast cancer cell lines (MF-F7 and HCC-1954), in Hacat, and in PBMC, by using the MTT method.

Results: The concentrations of total polyphenols in the ethanolic extracts of dry and fresh leaves of A. muricata were 803.75 and 422.50 μg/mL respectively. The phytochemical evaluation indicated the presence of several groups of compounds as shown in Table 1. No saponin was detected. It was observed that the extracts obtained from the dry leaves presented higher levels of flavonoids and anthraquinones than the extracts prepared from fresh leaves. The MTT test showed that both extracts from fresh and

Table 1 Phytochemical analysis of dry and fresh leaves of Annona muricata L.

Compound	*Dry leaves*	*Fresh leaves*
Alkaloides	++	++
Coumarins	+++	+++
Steroids	++	++
Reducing sugars	++	++
Fenoles y tannins	++	++
Anthraquinones	+++	++
Flavonoids	+++	++
Saponins	-	-

(-) no compound detected, (+) low levels (++) presence, (+++) high levels

dry leaves of A. muricata showed cytotoxic activity in the MF-F7 and HCC-1954 breast cancer cell lines, in Hacat cells and in PMNC; the cytotoxic activity was clearly doses-dependent (Figures 1 and 2). Interestingly it was observed that at 250 µg/mL both extracts were capable of inhibiting breast tumor cells proliferation, but not the proliferation of Hacat cells and PBMC, which indicates that A. muricata may present specificity against tumor cells.

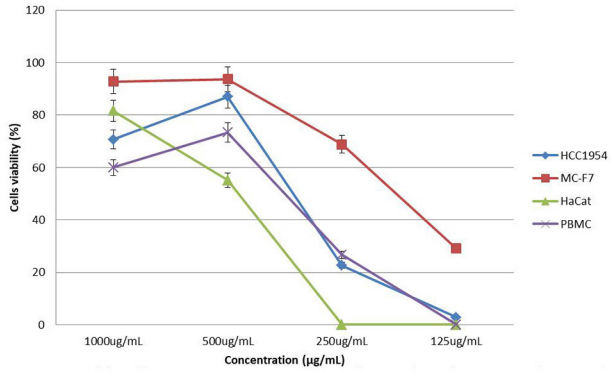


FIGURE 1: Viability of HCC1954, Me-Fl, He-Cat cell lines and PBMC after 48 h treatment with EFLAM of A. *muricata*

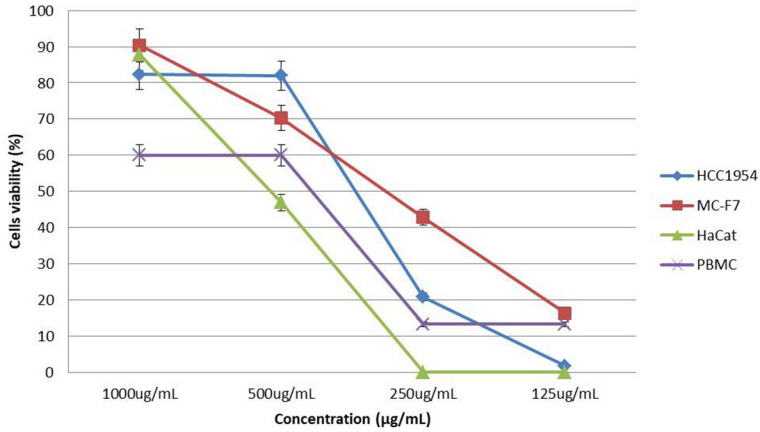


FIGURE 2: Viability of HCC1954, Me-FI, Ha-Cat cell lines and PBMC after 48 h treatment with EDLAM of A. *muricata*

Conclusions: The present results indicate that the ethanolic extracts from fresh and dry leaves of *Annona muricata* L., present cytotoxic activity, with interesting specificity towards breast cancer cell lines and may be a good candidates for further therapeutic applications. More investigation has been undertaken in order to get new knowledge of some of the possible mechanisms of action of this traditional medicinal plant and further characterize its antineoplastic activity.

REFERENCES

Organización Mundial de la Salud. (2017). Cáncer. [online] Available at: http://www.who.int/mediacentre/factsheets/fs297/es/ [Accessed 6 Dec. 2017].

Najmuddin S. et al. (2016). "Anti-cancer effect of Annona Muricata Linn Leaves Crude Extract (AMCE) on breast cancer cell line." BMC complementary and alternative medicine 16(1): 311.

Minari J. and Okeke U. (2014). Chemopreventive effect of Annona muricata on DMBA-induced cell proliferation in the breast tissues of female albino mice. Egyptian Journal of Medical Human Genetics, 15(4), pp.327-334.

Vainio H. and Bianchini F. (2002). Breast cancer screening. Lyon: International Agency for Research on Cancer, pp.119-121.

Angulo M. M. et al. (2013). Cáncer de mama. Medicine-Programa de Formación Médica Continuada acreditado, 11(27), 1629-1640.

Muñoz A. M. and Ramos F. (2007). "Componentes fenólicos de la dieta y sus propiedades biomedicinales." Horizonte Médico 7(1).

Kim G.T., et al. (2016). "Immunomodulatory efficacy of standardized Annona muricata (Graviola) leaf extract via activation of mitogen-activated protein kinase pathways in RAW 264.7 macrophages." Evidence-Based Complementary and Alternative Medicine 2016.

Coria A. V., et al. (2016). "Annona muricata: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity." Arabian Journal of Chemistry.

IL-2 rescues peripheral blood mononuclear cells from apoptosis induced by cervical cancer cells HeLa and Caski

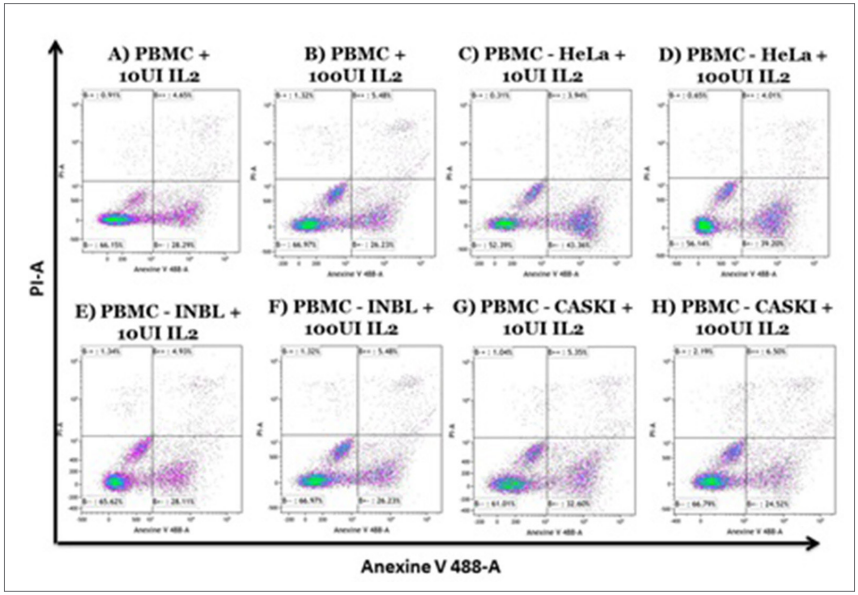
Adriana Gutiérrez-Hoya, Octavio Zerecero-Carreón, Arturo Valle-Mendiola, Benny Weiss-Steider, Isabel Soto-Cruz

National Autonomous University of Mexico, Cuernavaca, Mexico
adrianagh85@hotmail.com; octaviozerecero@gmail.com; arturo.valle@unam.mx; bennyweiss@hotmail.com; sotocruz@unam.mx

Keywords: Apoptosis, Lymphocytes, cervical cancer, IL-2, co-culture

Introduction: Cervical cancer is one of the world's deadliest diseases, is the fourth most common cancer in women. There were an estimated 530,000 new cases of cervical cancer in 2012; this represents 7.5% of female cancer mortality. Almost nine out of ten (87%) cervical cancer death occur in the developed countries, such as México. In the developed countries, the limited access to effective cervical cancer screening and HPV testing increases the mortality rate since the cancer is not detected until the symptoms appear in the more advanced phases. Additionally, the expectation of treatment is not good in advanced stages of the disease, for this reason, the mortality rate for cervical cancer is much higher than initially estimated (52%). Therefore it is necessary to study new molecules that can eliminate the tumour cells and that can activate the immune system. In this context our working group has found that high concentrations of IL-2 (Lymphocyte grown factor), promote the immune system activation and inhibited the growth of cervical cancer cells. Also, that the JAK/STAT pathway is active in cervical cancer cells. To understand the signalling pathways that are activated by IL-2 in cervical cancer cells that induce apoptosis and its relationship with normal immune cells we analysed the effect of high concentrations of IL-2 in co-cultures of cervical cancer cells and normal heterologous peripheral blood lymphocytes.

Methodology: Normal peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation using ficoll-paqueTM. The cervical cancer cell lines HeLa and Caski were co-cultured with the PBMC in a 1:3 ratio for 48 hours in the presence of different concentrations of IL-2. After this time the cells were stained with annexin V-PE and propidium iodide (PI) or 7AAD and analysed by flow cytometry.



Results: We observed that co-culturing cervical cancer cells with the PBMCs in the presence of low doses of IL-2 induced apoptosis in PBMCs (42%) and cervical cancer cells (35.5%). On the contrary, the co-culture of cervical cancer cells with the PBMCs in the presence of high doses of IL-2 rescued the PBMCs from apoptosis (35%), this percentage of apoptosis is similar to basal values (32%).

Conclusion: The cervical cancer cells can induce apoptosis in PBMCs. Nevertheless, high doses of IL-2 can rescue the PBMCs from undergoing apoptosis. It is possible that IL-2 is activating the JAK/STAT pathway to induce cell survival.

Expression of GPER in cervical cancer and its precursor lesions: Effect of its stimulation on the viability of cell lines derived from this cancer

Christian David Hernández Silva[1], Annie Riera Leal[1], Luis Felipe Jave Suárez[2], Pablo César Ortiz Lazareno[2], Jorge Adrián Ramírez De Arellano Sánchez[1], Mariel García Chagollan[1], Ana Laura Pereira Suárez[1]

[1]Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
[2]Centro de Investigación Biomédica de Occidente (CIBO), Guadalajara, Mexico
hernandezsilva7@gmail.com; annierieraleal78@yahoo.es; lfjave@gmail.com; pablolazareno@gmail.com; adrian.ramirezdearellano@hotmail.com; maye_999@hotmail.com; analauraps@hotmail.com

Keywords: Apoptosis, Immunohistochemistry, cellular proliferation, cervical cancer, senescence, estrogen, GPER, G-1

Introduction: Cervical cancer (CC) is one of the leading causes of death worldwide by cancer in premenopausal women. It is known that the human papillomavirus infection is necessary but not enough for its development, since it requires the cooperation of cofactors, such as the estrogen and their receptors [1,2]. Recently the G protein-coupled estrogen receptor (GPER) has been studied in a variety of cancers, including breast, ovarian and endometrium cancer [3]. Also, the expression of GPER was reported in CC cell lines (HeLa, SiHa and C-33A) and in CC tissue samples [4,5]. The purpose of this study was evaluate the expression of GPER in different degrees of cervical lesions and whether the stimulation with its specific agonist (G-1) modulated cell survival in CC cells and in a non-tumorigenic keratinocytes HaCaT cells.

Materials and Methods: GPER expression in CC and its precursor lesions was analyzed by automated immunohistochemistry. CC cell lines (HeLa, SiHa and C-33A) and HaCaT, were stimulated with G-1 (1 μM) and the GPER- selective inhibitor G15. Changes in the mitochondrial membrane potential were evaluated by immunofluorescence using the MitoCaptureTM (Cat. K250, BioVision) Kit. Cell proliferation was analyzed in real time using the xCELLigence RTCA Systems (ACEA Biosciences, Inc.), while apoptosis was determined by flow cytometry using FITC Annexin V/Dead Cell Apoptosis (Cat. V13242, InvitrogenTM) Kit. Senescence assay was evaluated by an optical microscopy using a senescence kit (Cat. K320, BioVision).

Result: GPER expression increased in CC samples during the progression to cancer. The staining is mostly cytoplasmic but is also observed at the level of the cell membrane and in the nucleus of the tumor cells. G-1 inhibited the proliferation rate of the CC cell lines but only in HeLa and in C33A it induced apoptosis. In SiHa G-1 increased the degree of cellular senescence. An early modification of the mitochondrial membrane potential it was observed in all cell lines.

Conclusions: GPER is an estrogen receptor associated with CC. Specific stimulation with G-1 decreases the proliferation index of the cell lines derived from this cancer through the modulation of the mechanisms of apoptosis and cellular senescence.

REFERENCES

1. Bronowicka-Klys, DE, Lianeri M, Jagodzinski PP. The role and impact of estrogens and xenoestrogens on the development of cervical cancer. Biomed Pharmacother (2016) 84: 1945-1953. doi: 10.1016/j.biopha.2016.11.007
2. Chung SH, Wiedmeyer K, Shai A, Korach KS, Lambert PF. Requirement for estrogen receptor alpha in a mouse model for human papillomavirus-associated cervical cancer. Cancer Res (2008) 68(23): 9928-9934. doi: 10.1158/0008-5472.CAN-08-2051
3. Jacenik D, Cygankiewicz AI, Krajewska WM. The G protein-coupled estrogen receptor as a modulator of neoplastic transformation. Mol Cell Endocrinol (2016) 429: 10-18. doi: 10.1016/j.mce.2016.04.011
4. Zhang Q, Wu YZ, Zhang YM, Ji XH, Hao Q. Activation of G-protein coupled estrogen receptor inhibits the proliferation of cervical cancer cells via sustained activation of ERK1/2. Cell Biochem Funct (2015) 33(3): 134-142. doi: 10.1002/cbf.3097
5. Friese, K, Kost B, Vattai A, Marme F, Kuhn C, Mahner S, Dannecker C, Jeschke U, Heublein S. The G protein-coupled estrogen receptor (GPER/GPR30) may serve as a prognostic marker in early-stage cervical cancer. J Cancer Res Clin Oncol (2018) Jan;144(1):13-19. doi: 10.1007/s00432-017-2510-7

Regulation of IL-6 expression by E5, E6 and E7 proteins from high and low risk human papillomaviruses

Cristina Artaza Irigaray, María Guadalupe Flores Miramontes, Pablo César Ortiz Lazareno, Eliza Julia Pedraza Brindis, Adriana Aguilar Lemarroy, Luis Felipe Jave Suarez

Centro de Investigación Biomédica de Occidente (CIBO), Guadalajara, Mexico
chispuncris@hotmail.com; nereyda_2404@hotmail.com; pablolazareno@gmail.com; elabrindis@gmail.com; adry.aguilar.lemarroy@gmail.com; lfjave@live.com.mx

Keywords: Human papillomavirus, IL-6, Cytokine expression, E6 protein, high-risk and low-risk HPVs

High-risk human papillomaviruses (HPV) are the main causative agents for cervical cancer (CC) development. They evade the host´s immune response and contribute to keratinocyte transformation through E5, E6 and E7 oncoproteins. IL-6 cytokine is expressed in CC cell lines infected with HPV16 and HPV18 but is not found in HPV negative C33A CC cell line. The aim of this work was to determine if E5, E6 and E7 viral proteins from HPVs 16, 18, 62 and 84 differentially regulate IL-6 expression. First, the non-tumorigenic keratinocyte cell line HaCaT was chosen as a study model to establish 12 HaCaT subclones by genome integration of E5, E6 and E7 viral genes from high-risk HPV16 and HPV18 and from low-risk HPV62 and HPV84 using a lentiviral expression system. We showed that IL-6 cytokine is overexpressed in CC derived cell lines compared to HaCaT cells, IL-6 mRNA expression increased in HaCaT cells transduced with HPV16, 18 and 62 E6 but not with HPV84 E6 and E7 slightly raises IL-6 expression while E5 does not. Moreover, Western Blot, LEGENDplex Human Inflammation panel and ELISA tests confirmed the increase in IL-6 protein production and release by E6 proteins. Finally, the regulation of chemokines and cytokines by E6 proteins was determined by expression microarrays and qPCR showing that high risk E6 proteins downregulate CXCL10 and CCL17 expression, and upregulate CCL26 and CXCL3. In this work we demonstrated that E6 proteins, mainly from high-risk HPVs, promote IL-6 overexpression and regulate the expression of some chemokines and cytokines in keratinocytes, promoting a microenvironment prone to tumorigenesis.

Cross-hybridization between alpha-3 HPV genotypes in the Linear Array Genotyping Test

Cristina Artaza Irigaray[1], María Guadalupe Flores Miramontes[2], Dominik Olszewski[2], Verónica Vallejo Ruiz[3], Laura Patricia Limón Toledo[4], Cibeles Margarita Ciboney Sánchez Roque[1], Rocío Mayoral Torres[1], Luis Felipe Jave Suárez[1], Adriana Aguilar Lemarroy[1]

[1]Centro de Investigación Biomédica de Occidente (CIBO), Guadalajara, Mexico
[2]Heidelberg University of Education, Heidelberg, Germany
[3]Centro de Investigación Biomédica de Oriente (CIBIOR)
[4]Centro Médico Nacional de Occidente (IMSS), Guadalajara, Mexico
chispuncris@hotmail.com; nereyda_2404@hotmail.com; dominik.ol190592@gmail.com; veronica_vallejo@yahoo.com; patricialimontoledo@gmail.com; ciboneyroque@gmail.com; rosi-2408@outlook.es; lfjave@yahoo.com; adry.aguilar.lemarroy@gmail.com

Keywords: Human papillomavirus, Next-generation sequencing, Linear Array Genotyping Test, Cervical samples, L1 mutations

Linear Array Genotyping Test (LA) is one of the gold standards used for HPV genotyping, however, Next-Generation Sequencing (NGS) allows genotyping of a broader spectrum of HPVs with a high specificity. In this study, using double stranded L1 fragments (gBlocks) from different HPVs to perform LA test, we showed that there is a cross-hybridization between alpha3-HPV genotypes 86, 87 and 114 with HPV84 probe in LA strips and between HPV102 with HPV83 probe. To corroborate this observation, 14 HPV83+ and 26 HPV84+ cervical samples determined with LA, were individually genotyped by NGS, revealing that from the LA HPV83+ samples, 64.3% were truly HPV83+, while 42.9% were HPV102+. Moreover, only 69.2% of the LA HPV84+ samples were HPV84+, while 3.8%, 11.5% and 30.8% of the samples were indeed HPV 86, 87 and 114 positive, respectively. Finally, novel nucleotide changes in L1 gene from HPVs 83, 84, 87, 102 and 114 were determined in Mexican cervical samples, some of them lead to changes in the protein sequence. In the upcoming years, a switch to more specific and sensitive genotyping methods that detect a broader spectrum of HPV genotypes is essential to unravel the biological and clinical significance of HPV coinfections.

Crosstalk between NF-kappaB and the HOXA9 transcription factor and its relation with immune respond in Cervical Cancer derived cells

Liliana Alvarado-Ruiz[1], Cibeles Sánchez-Roque[2,3], Rosio Mayoral-Torres[2], Adriana Aguilar-Lemarroy[2], Luis Felipe Jave-Suarez[2]

[1]Universidad del Valle de México, Mexico City, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Hospital Civil de Guadalajara, Guadalajara, Mexico
lilit1585@hotmail.com; ciboneyroque@gmail.com; rosi-2408@outlook.es; adry.aguilar.lemarroy@gmail.com; lfjave@gmail.com

Keywords: Neoplasms, cervical cancer, NF-kB pathway, Crosslinking, HOXA9

Cervical cancer (CC) is one of the main neoplasms worldwide, it is associated with infection by human papillomavirus (HPV) and other factors. HOXA9, a transcriptional regulator has been observed to be decreased in cells derived from CaCU. HOXA9 interacts with the NFkB pathway, however its relationship in CaCU is not clear, which is addressed in this work

Objective: To evaluate the influence of HOXA9 expression on NF-κB canonical and non-canonical pathways and immune response associated molecules on non-tumorigenic keratinocytes and cervical cancer (CC) derived cells.

Methods: Non-tumorigenic keratinocytes and CC derived cell lines were used as comparative models. All cells were cultured in DMEM at 37°C and 5% CO2. Of each cell lines, mRNA was extracted and used to perform qRT-PCR experiments. Primers for HOXA9 and target genes implicated on canonical and non-canonical NF-κB pathway were designed: p100, p105, p65, C-REL, REL-B, IkBa, cMyc, HDAC2, Bcl-xL, Bcl-2, Bax, Bad, Bak, BIRC-2, Caspasas 3, 8 y 9, VCAM, ICAM, MMP7, AGTR-1, p21 e HIF1-a. HOXA9 open reading frame was cloned and introduced into the HeLa cell line by means of lentiviral expression system. HOXA9 overexpression was corroborated by qPCR. Afterwards, the effect of HOXA9 on NF-κB pathway activation, cell proliferation and apoptosis was evaluated in the established cell lines. Variations in mRNA level of the selected target genes were quantified and correlated with HOXA9 expression.

Results: NF-kB Pathway. In the cells derived from CC we found an overexpression of the components of the non-cannonical NF-kB pathway, such as Rel-B and p100 (7 and 2.7 times more in comparison to the non-tumorigenic queratinocytes, p= 0.001 and *p*=0.0005). Also, the subunits of the cannonical pathway were found to be under-expressed in cells derived from CC, such is the case of p65 which was found 0.81 times underexpressed in HeLa cells than in HaCat cells ($p<0.05$), p105 was 0.16 times under expressed in CC (p=0.0002) and cRel 0.10 times less in comparison to non tumorigenic queratinocytes (p=0.001). In addition, the regulator of the NF-kB pathway: IkBa, was found to be underexpressed as well in CC up to 0.32 times less than HaCaT cells (p=0.0004). By the other hand, among cells in which we restored the expression of HOXA9 we found an overexpression of all the components of the cannonical pathway of NF-kB, such as p65 (Rel-A) with 1.47 times more than HeLa-LVx cells (p=0.0002), p105 with 1.47 times more (p=0.0007) and C-Rel with 1.11 times more in comparison to the cells derived from CC without restoring the expression of HOXA9, although in this last case there were no significant differences (p=0.095). As well, we found the principal components of the non-cannonical pathway to be overexpressed up to 16 times more, in case of Rel-B (p=0.0002) and 6.67 times more for p100 (p=0.0003) in the HeLa-HOXA9 cell line. Nonetheless, the regulator IkBa was found to be under expressed when restoring the expression of HOXA9, 37 times less than tumorigenic HeLa-LVx cells (p=0.0013).

Genes involved in apoptotic regulation. We chose to evaluate modulator genes of apoptosis from the Bcl-2 family, finding an under-expression in the cells derived of CC of the regulators Bcl-2 and Bcl-xL of 0.15 and 0.32 times les, respectively, than the non-tumorigenic keratinocytes (p=0.0004 and p=0.0002, respectively). As well, the Bax an Bad molecules were found to be under-expressed 0.71 and 0.45 times less in the CC cells in comparison to the non-tumorigenic control (p=0.004 and p=0.0008 respectively). On the contrary, the Bak molecule was found to be over-expressed in the tumor cells in comparison to the normal keratinocytes up to 8.87 times more (p=0.0001). These members of the Bcl-2 family were all showed to be over-expressed once that the expression of HOXA9 was reestablished in the tumor cells; the anti-apoptotic molecules Bcl-2 and Bcl-xl were found to b over-expressed 1.5 and 2.28 times more in the HeLa-HOXA9 cell line than in the HeLa-LPX control line (p=0.007 and *p*=0.0009 respectively). In other molecules of this family, such as Bax, Bad and Bak, we also found an over-expression of 6.83, 8.42 and 1.06 times more than in Hela-LVx even if the latter of the three has no statistically meaningful effect (p=0.0003, p=0.0003 and p=0.21 respectively). We also evaluated the effect of the restoration of the genetic expression of HOXA9 on the expression of the apoptosis inhibitor BIRC2, which was found to be under-expressed in the tumorigenic cell line up to 0.69 times less than HaCat ($p<0.0001$) and over-expressed by then restoring the expression of HOXA9 (2.07 times more than the HeLa-LVX control line with p=0.002).By evaluating the

expression of the caspases involved in the intrinsic as well as the extrinsic pathways, we found an under-expression of them in the cells derived from CC, 0.14 times less in the caspase 3 and 0.10 times less in the Caspase 8 in comparison to HaCat (p=0.0001 and p=0.0001), but there was also found a modest increase in the expression of Caspase 9 in the tumor cells (1.04 times in relation to the non-tumorigenic keratinocytes) this increase was not statistically meaningful (p=0.208).

In contrast, the cells in which we restored the expression of HOXA9 we found an over-expression of all of them, up to 2.16 times more in the caspase 8 in the HeLa-HOXA9 (p=0.0021), 1.19 times in caspase 3 in comparison to HeLa-LVX (p=0.036) and 1.37 times more in Caspase 9 in HeLa-HOXA9 cells in comparison to HeLa-LVX cells.

Conclusions: The restoration of HOXA9 expression in CC derived cell lines probably induces the stabilization of the non-canonical NF-kB pathway and a decrease in the canonical pathway, as a result of the IkB-A increase and p100 and REL-B decrease. These observations could be related to the mesenchymal to epithelial phenotype change in the cells tumor, observed when restoring the expression of HOXA9 in HeLa cells. Studies at the protein level are necessary to understand more precisely the interrelation between HOXA9, NFkB and the biological processes involved in tumor progression.

Contributions of the CD43 sialomucin to shape the tumurogenic environment: The secretion of factors that modulate the tumorigenic process in A549 cells

Daniela Vega Mendoza[1,2,3], Alicia Cañas Linares[1,2,3], Yvonne Rosenstein[2,3]

[1]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[2]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico
[3]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
Dvega@ibt.unam.mx; linares@ibt.unam.mx; yvonne@ibt.unam.mx

Keywords: Angiogenesis, tumor cells, Secretome, tumoral microenvironment, CD43

CD43 is a glycoprotein expressed by immune cells and tumor-derived cell lines from lung carcinoma, cervix, colon, and breast. We and others have provided evidence that CD43 delivers signals that enhance the tumorigenic capacity of non-hematopoietic tumor cells. However, the molecular mechanisms responsible for that are not entirely understood. We had previously shown that decreasing CD43 expression by RNA interference in A549 cells (lung adenocarcinoma) impaired their motility, anchorage-independent growth as well as in vivo tumor formation. The goal of this study is to evaluate the possible role of CD43 in the secretion of molecules that promote the tumoral capacity of A549 cells as well as molecules that modulate tumor-related immune responses. We analyzed the secretome from 48hr-starved A549 cells with high and low CD43 expression by LC-MS/MS and identified 631 proteins out of which 143 were differentially expressed. Out of these, 48 proteins were up-regulated in CD43 high cells, and 51 were up-regulated in CD43 low cells. Among the proteins that were overexpressed by the A549 CD43hi cells, we found fibrinogen, one of the most important coagulation factors and that has been shown to be a critical determinant of the metastatic potential of circulating tumor cells in lung carcinoma and melanoma. Furthermore, elevated pre-treatment plasma fibrinogen levels have been associated with poor clinical outcome in various malignancies. With an antibody-based array, we screened for cytokines released to the culture supernatant, and we found that CD43 high cells produced higher amounts of IL-8 and MCP-1 ($p < 0.05$) as compared with CD43 low cells. The bioinformatics analysis of the differentially expressed proteins revealed that the most enriched biological processes were "angiogenesis", "extracellular matrix organization", "cell adhesion", "protein glycosylation", "cell-cell adhesion",

"cell proliferation", "collagen catabolic process" and "cell migration". Based on these results, we measured the angiogenic capacity of the secretome from cells with different expression levels of CD43. We found that the secretome of cells with higher levels of CD43 produced more vessel sprouting than that of cells with lower levels CD43 ($p < 0.05$). Altogether this data underscores novel functions for the CD43 molecule for tumor progression. Whether this extends to lymphoid cells and lymphoid cells tumors remains to be elucidated.

Interplay between high risk HPV oncoproteins with estrogen and prolactin: Effects on the hormonal receptors expression, cell metabolism and the cytokine profile

Inocencia Guadalupe Ramírez López[1], Mariel Garcia Chagollan[1], Luis Felipe Jave Suarez[2], Annie Riera Leal[1], Jorge Adrián Ramírez de Arellano Sánchez[1], Cristina Artaza Irigaray[1,2], Ana Laura Pereira Suárez[1]

[1]Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
ino_141992@hotmail.com; maye_999@hotmail.com; lfjave@gmail.com; annierieraleal78@yahoo.es; adrian.ramirezdearellano@hotmail.com; chispuncris@hotmail.com; analauraps@hotmail.com

Keywords: Prolactin, cervical cancer, HPV, 17β-estradiol, PrlR, ERα, ERβ, E7 oncogene, E6 oncogene

Introducction: Cervical cancer (CC) is the second most common cancer in less developed countries and the second leading cause of death in women worldwide. The 99% of patients are infected with the Human Papilloma Virus (HPV), being the most frequent the HPV16 and HPV18 infection. Even though it is considerate a necessary factor for the development of CC, it is not enough, since it has been shown that HPV requires the participation of other cofactors, such as the hormonal ones. Several studies have demonstrated the requirement of estrogen (E) and its receptors (ERα, ERβ) in the progress towards CC of the precursor lesions. Also, prolactin (PRL) and its receptor (PRLR) have been associated with CC.

The molecular mechanisms underlying the cooperation of these hormones with the viral oncoproteins are not well elucidated, that is why in this study we evaluated the effects of the stimulation with E and PRL on the expression of the E6 and E7 oncogenes and the hormonal receptors REα, REβ and PRLR; in addition the impact on the cellular metabolism and in the cytokine profile produced by transfected line of non-tumorigenic keratinocytes HaCaT cells were determined.

Material and Methods: PCR was used to evaluate the effect of E and PRL on the expression of E6 and E7 oncoproteins in CC cells lines. HaCaT cells were transduced with the viral oncogenes E6 and E7 of HPV 16 and 18 and were stimulated with 17β-estradiol (10nM) and PRL (200ng/mL). The cell metabolism was evaluated by MTT assay at the

72 hours. The cytokines profile was determined by MULTIPLEX, while the expression of ERα, ERβ and PRLR was assessed by the Western Blot technique.

Results: 17β-estradiol and PRL induced an increase in E6 and E7 mRNA expression of HeLa cell line. Both hormones elevated the cellular metabolism in HaCaT cells transduced with HPV 18-E6 and HPV 16-E7 oncogenes. The expression of the HPV 16-E6 induced a rise in the production of G-CSF, IL-7, IL-8, MCP-1, INFγ, IL-2, IL-6, TNFα and IL-4. The treatment with 17β-estradiol enhanced the levels of IL-7, INF-γ, TNF-α and IL-4 while with PRL only the G-CSF, IL-7 and IL-4 were increased. E6 of both HPV 16 and 18 stimulated the expression of ERβ and PRLR but not ERα whereas E7 only had this result in the expression of PRLR.

Conclusions: Estrogen and prolactin cooperate with the viral oncogenes E6 and E7 to induce the carcinogenesis process in cervical cancer through the modulation of the expression of the viral oncogenes, of the hormonal receptors and of affecting the cellular metabolism and the profile of cytokines.

The CD43 sialomucin promotes tumor cell survival and tumor growth

Alicia Cañas Linares[1,2], Angel Francisco Flores Alcantar[1,2], Daniela Vega Mendoza[1,2], Yvonne Rosenstein[1,2]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
linares@ibt.unam.mx; alcantar@ibt.unam.mx; dvega@ibt.unam.mx; yvonne@ibt.unam.mx

Keywords: lung cancer, Tumor growth, cell survival signaling, CD43 sialomucin, non-lymphoid tumor

CD43 has long been considered as a leukocyte-restricted molecule. Accordingly, it is expressed in more than 90% of T-cell lymphomas and T-cell lymphoblastic lymphomas as well as in more than 50% of B-cell lymphoblastic and low-grade B-cell lymphomas. In addition to hematopoietic tumors, non-hematopoietic, tumor-derived cells from the breast, lung, colon, bladder, cervix, and prostate express CD43. CD43 expression by tumor cells is associated with an adverse prognosis as CD43 has been described to participate in tumor cell adhesion and motility as well as to control cell cycle entry, ultimately favoring cell transformation.

We reported recently that in lung, colon, and cervix tumor cells, CD43 cooperates with oncogenic signals to promote cell transformation by abrogating the contact inhibition of growth through a molecular mechanism that involves the AKT-dependent Merlin phosphorylation and degradation (1). To further characterize the role of CD43 in cell transformation, we evaluated the response of the acute T cell leukemia Jurkat cells and the lung carcinoma cell line A549 to different culture conditions. Our principal goal was to assess the participation of the CD43 molecule in the regulation of signaling pathways promoting proliferation and survival. We used cells expressing normal levels of CD43, cells where CD43 expression level was inhibited by RNA interference or cells expressing a form of CD43 lacking the intracellular domain. We will present data suggesting that CD43 expression is associated with cell survival under stress conditions by regulating the expression level of different molecules involved in apoptosis. Furthermore, we found that CD43 expression favors non-metabolic functions of the glycolytic enzyme PKM2, as well as it participates in the regulation of the expression of nutrient receptors. This data underscores the role of the CD43 sialomucin in both, lymphoid and non-lymphoid tumor progression.

REFERENCE

1. (PLoS ONE 8(11): e80806. doi:10.1371/journal.pone.0080806).

The IL17RA/IL17 pathway sustain the induction of effector and memory cytotoxic T cell responses against tumors

Constanza Rodriguez, Jimena Tosello Boari, Cintia Araujo, Fernando Canale, Cristian Gabriel Beccaria, Sabrina Bossio, Santiago Boccardo, Adriana Gruppi, Carolina Montes, Eva V Acosta Rodriguez

Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI CONICET), Cordoba, Argentina
coti.r@hotmail.com; Jtosello@fcq.unc.edu.ar; Caraujo@fcq.unc.edu.ar; Fcanale@fcq.unc.edu.ar; Cbeccaria@fcq.unc.edu.ar; Sbossio@fcq.unc.edu.ar; Sboccardo@fcq.unc.edu.ar; Agruppi@fcq.unc.edu.ar; Cmontes@fcq.unc.edu.ar; Eacosta@fcq.unc.edu.ar

Keywords: Vaccination, CD8+ T cells, IL-17, tumor immunity, Li T activation

IL-17 cytokines play important roles in protecting the host against extracellular pathogens and promoting inflammatory pathology in autoimmune diseases (1). In cancer, several reports have shown that IL-17 can have both anti- and pro-tumoral effects (2). However, the particular mechanism that determines its tumor promoting or suppressive functions in a given tumor are not known yet. Previously, our team and others demonstrated that IL-17 family plays a central role in the induction of CD8+ T cell (CTL) and NK responses (3). As these subsets are critical for tumor resistance, we evaluated the role of IL-17 signaling in the induction of the anti-tumoral CTL immunity and tumor progression. To this end, B6 (WT), IL-17RA KO (RKO) and IL-17A/F double KO (DKO) mice were injected with tumor cell lines exhibiting progressor (B16-SIY and MCA101-OVA) and regressor (MC57-SIY) growth patterns (4, 5). We determined that volumes of B16 and MC57, but not MCA101, tumors were increased in RKO and DKO mice compared to WT mice at several days post injection (dpi). To further understand these differences in tumor progression, we evaluated the primary CTL response against B16 and MC57 tumors. We determined that 20 days upon B16 injection, RKO and DKO mice showed reduced numbers of total and tumor-specific CD8+ T cells ($p<0.05$) and increased expression of several inhibitory receptors (PD1, LAG3, TIM3) ($p<0.05$) within tumor infiltrating lymphocytes in comparison to WT counterparts . Similarly, analysis of the primary CTL response against MC57 demonstrated that compared to WT controls, DKO mice presented reduced numbers of tumor-specific CTL in draining-lymph nodes at d12pi ($p<0.05$). Also, tumor-specific CTL displayed decreased numbers of cells with memory phenotype (CD62L+CD127+) ($p<0.05$). As the memory CTL response developed in MC57-SIY-immunized WT mice is critical for host protection against challenge with B16-SIY tumors, we evaluated whether RKO and DKO mice were able to generate protective SIY-specific CTL memory. To this end,

we challenged MC57-SIY immunized mice with B16-SIY tumor cells and determined tumor growth until day 32 post challenge (pc). While most of immunized WT mice (75%) rejected the B16 tumors, immunized DKO and RKO mice were less protected as only few mice (around 20%) were tumor free at d32pc ($p<0.05$). Accordingly, blood and tumors of DKO mice showed reduced frequency of SIY-specific CTL ($p<0.05$) as well as decreased tumor-specific cells with a memory effector phenotype (CD44+CD62L-; $p<0.001$) in comparison to WT controls at day 8pc. Altogether, our results indicate that the IL-17/IL-17RA pathway modulates primary and secondary antitumor CTL responses and may influence tumor progression in certain cancers. Further studies on the role IL-17 signaling within the tumor microenvironment will provide rationale to understand the risks and benefits of targeting IL-17 in a given tumor.

REFERENCES

1. Song X, He X, Li X, Qian Y. The roles and functional mechanisms of interleukin-17 family cytokines in mucosal immunity. Cell Mol Immunol (2016) 13(4):418-31. doi: 10.1038/cmi.2015.105. PubMed PMID: 27018218; PubMed Central PMCID: PMC4947810.
2. Qian X, Chen H, Wu X, Hu L, Huang Q, Jin Y. Interleukin-17 acts as double-edged sword in anti-tumor immunity and tumorigenesis. Cytokine (2017) 89:34-44. doi: 10.1016/j.cyto.2015.09.011. PubMed PMID: 26883678.
3. Bar E, Whitney PG, Moor K, Reis e Sousa C, LeibundGut-Landmann S. IL-17 regulates systemic fungal immunity by controlling the functional competence of NK cells. Immunity (2014) 40(1):117-27. doi: 10.1016/j.immuni.2013.12.002. PubMed PMID: 24412614.
4. Spiotto MT, Yu P, Rowley DA, Nishimura MI, Meredith SC, Gajewski TF, et al. Increasing tumor antigen expression overcomes "ignorance" to solid tumors via crosspresentation by bone marrow-derived stromal cells. Immunity (2002) 17(6):737-47. PubMed PMID: 12479820.
5. Williams JB, Horton BL, Zheng Y, Duan Y, Powell JD, Gajewski TF. The EGR2 targets LAG-3 and 4-1BB describe and regulate dysfunctional antigen-specific CD8+ T cells in the tumor microenvironment. J Exp Med (2017) 214(2):381-400. doi: 10.1084/jem.20160485. PubMed PMID: 28115575; PubMed Central PMCID: PMC5294847.

Analysis of T-lymphocytes and their distinct subsets in lung adenocarcinoma patients previous and during treatment with conventional chemotherapy

Lorenzo Islas-Vazquez, Alfredo Florencio-Vargas, Dolores Aguilar-Cazares, Miriam Galicia-Velasco, Heriberto Prado-Garcia, Manuel Meneses-Flores, Uriel Rumbo-Nava, Jose Sullivan Lopez-Gonzalez

Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
lorenzo.islasv@gmail.com; alfredoflova@gmail.com; doloresaguilarcazares@yahoo.com.mx; miriam_galicia_velasco@yahoo.com.mx; hpradog@yahoo.com; manuelmeneses707@gmail.com; urumbo@gmail.com; slopezgonzalez@yahoo.com

Keywords: T lymphocytes, CTLs, Inflammatory infiltrate, Lung Adenocarcinoma, Chemotherapy

Background: Lung cancer is the leading cause of cancer death worldwide. According to the molecular profile of EGFR mutations, lung cancer patients are treated with TKIs when their tumors contain sensitive EGFR mutations. However, a high percentage of patients express the wide-type EGFR. In this case the choice treatment is in base to platinum compounds. This type of treatment directly impacts on the tumor cell viability. In addition, during tumor cell death, several chemotherapeutic compounds promote the release of damage-associated molecular patterns (DAMPs), alter some of tumor evasion mechanisms or reduce the proportion of some subpopulations of immune cells with immunosuppression activity. According to these sceneries, we consider evaluate, in lung cancer patients previous and during treatment, changes in the CD8+ and CD4+ T-lymphocytes and in some CD4+ T subsets. Also, relate these changes to patient´s survival.

Objective: Previous to therapy schedule, we detected and compared the percentages of CD8+ and CD4+ T-lymphocytes and some CD4+ T subsets between healthy subjects and lung adenocarcinoma patients. In lung cancer patients, changes induced by the chemotherapy in the proportion of the distinct subpopulations studied were quantified and variations were associated to patient´s survival. In addition, in the inflammatory infiltrate from patient's biopsies, localization and proportion of CD8+ T-lymphocytes were done.

Material and Methods: A total of 28 patients with advanced stages of lung adenocarcinoma treated with six cycles of chemotherapy were studied. PBMC were collected previous to and at the third and sixth cycles of treatment. As control groups, PBMC from 13 healthy non-smoking and 15 heavy-smoking volunteers were obtained. From PBMC, percentages of Th1 (T-bet+), Th2 (CRTH2+), Th17 (RORg+) and Treg (CD25+, CD127-) subpopulations were determined. Total CD8+ T-lymphocytes and the subpopulation granzyme B+ perforin+ were quantified. From the CD4+ and CD8+ lymphocytes, the naïve (CD45RO+CD27-), effector (CD45RO-CD27-), central memory (CD45RO+CD27+) and effector memory (CD45RO+CD27-) phenotypes were studied. In diagnostic biopsies from patients, the tumor infiltrating CD8+ T-lymphocytes were quantified. The parameters studied were associated with the patient's median average survival.

Results: Compared to both control groups, higher percentages of Th17 and Treg lymphocytes were quantified in lung cancer group. Also, higher proportions of CD4+ T-lymphocytes with effector phenotype were detected. In lung adenocarcinoma patients, the group with better median average survival (more than 12 months) showed higher percentages of total CD8+ T-lymphocytes with effector phenotype. This result was supported with the detection of CTLs co-expressing granzyme B and perforin. In biopsies, an immune excluded phenotype with high density of peritumoral CD8+ T-lymphocytes with a memory phenotype (CD45RO+ cells) were found.

Conclusions: In patients group, the increase of Th17 and Treg lymphocytes detected previous to treatment did not affect the percentage of CD8+ T lymphocytes. According the mean average survival of patients under treatment, higher percentages of CD8+ T-lymphocytes containing the cytotoxic molecules (effector phenotype) where detected previous to treatment, and a tendency to increase was detected in patients with better median average survival. Respect to cancer tissue analyses, high infiltration of memory CD8+ T-lymphocytes were observed. Our results suggest that, in the CD8+ T-lymphocytes, determination of the effector/memory/naive phenotype ratios may be used as a prognostic factor associated to lung adenocarcinoma patient´s survival. In addition, even in peripheral blood the effector CD8+T-lymphocyte subset is increased, infiltration of tumors is mainly by memory CD8+ T-lymphocyte subset. To impact in the lung adenocarcinoma patient´s survival, in addition to TKIs and immunotherapy treatments, further strategies must be developed for favoring migration of effector T-lymphocytes into tumor nests.

Role of the signal transducer and activation of transcription 6 in regulation of Wnt/β-catenin signaling pathway in early stages of Colitis-associated Colon Cancer

Lesly Casasola-Trejo, Sonia Andrea Leon-Cabrera

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
casasola_lesly@hotmail.com; soleon81@gmail.com

Keywords: Macrophages, STAT6, Wnt signaling, CAC, WNT2B

Colorectal cancer is a disease with high prevalence worldwide that presents a late diagnosis with radical therapeutic options that contribute to a poor quality of life in patients (1). It is well known that inflammatory bowel diseases favors the development of cancer due to several factors (2, 3). There is evidence that alternative activated macrophages (M2) which have an important role in wound healing, are able to secrete Wnt ligands in a STAT6 dependent manner (4). However, it is unknown whether these ligands are associated with an upregulation of the Wnt / β-catenin pathway related to the characteristic uncontrolled proliferation of cancer.

To evaluate if these ligands are related to the activation of the Wnt / B-catenin pathway in early stages of colitis-associated colon cancer (CAC), we used a murine model of CAC in wild type (WT) and STAT6-deficient mice (STAT6- / -), in which the rate of disease damage and weight was measured twice per week during the experiment. Sacrifices were performed in early and late stages of tumor development in which the presence of tumors in the colon was evaluated macroscopically. Samples were obtained and mRNA was extracted from the colon tissue where RT-PCR was performed to identify the presence of classical macrophages (iNOS), alternatively activated macrophages (Fizz, Arginase), wnt2b ligand, the activation of the wnt B-catenin pathway by c-Myc and Lgr5 as well as genes to evaluate cytokine expression (IL-10, TNF-alpha). Also, in the early stages of tumor development, lamina propria macrophages were obtained and the presence of characteristic genes of the M1 and M2 phenotype were analyzed, besides the Wnt2b ligand and the proliferation gene c-Myc. We observed that STAT6 -/-mice showed weight gain, a lower score in the activity disease index and they developed fewer and smaller tumors compared whit WT mice. In the macrophages obtained from the lamina propria in the early stages of CAC, it was observed that in STAT6 deficiency there was an overexpression of wnt2b in comparison with the WT group. Furthermore, in intestinal tissue, there was a higher expression of Lgr5 and c-Myc in

STAT6 -/- mice compared with WT animals. Our results suggest that STAT6 mediates the expression of Wnt2b ligand during early stages of CAC development. Activation of wnt signaling in STAT6-dependent manner could promote mucosal repair during chronic inflammation and prevent tumor development.

REFERENCES

1. Grivennikov et al; Inflammation an colorrectal cáncer: colitis-associated neoplasia. Semin Immunophatology. 2013 March: 35(2):229-244.
2. Terzic et al. Inflammation and colorrectal cáncer, Gastroenterology (2010) Vol.138, No6. pp2101-2114.
3. León-Cabrera et al; Lack of STAT6 attenuates inflammation and drives protection against early steps of colitis-associated colon cáncer, Cancer Immunology Research, 2017.
4. Cosin-Roger et al; The activation of Wnt signaling pathway by a STAT6-dependent macrophage phenotype promotes mucosal repair in murin IBD. Mucosal Immunology. Nature Publishing group. 2015. pp1-13.

Essential oil of Eugenia uniflora as a potential immunomodulator in tumoral response

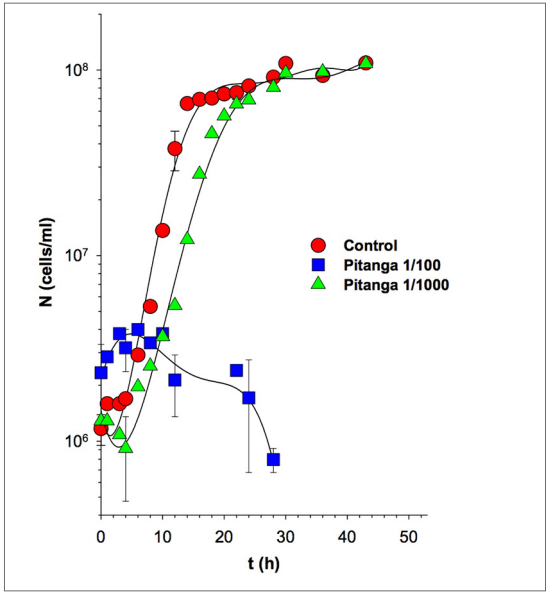
Gabriela Ferragut[1], Ana Gabriela Sánchez[1], Pamela Lombardo[1], Juan Cedano[1], Beatriz Vignale[1], Eduardo Dellacassa[2], Deborah Juana Keszenman[1]

[1]University of the Republic, Montevideo, Uruguay
[2]Facultad de Química, Universidad de la República, Montevideo, Uruguay
gferragu@fq.edu.uy; anagab77@gmail.com; palomba@adinet.com.uy; jcedano@unorte.edu.uy; herbea@vera.com.uy; edellac@fq.edu.uy; dkeszen@gmail.com

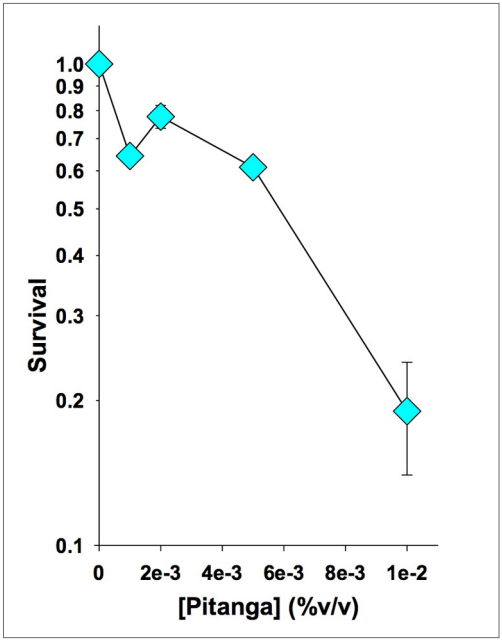
Keywords: colorectal cancer, proliferation, yeast, essential oils, Eugenia uniflora

Introduction: It is estimated that 80% of the inhabitants of the earth depend on Folk or Traditional Medicine for their primary care needs. This type of healthcare mostly relies on treatments that use plants or their derived products. In developed countries, medicine based on the use of natural products has gained popularity in part because of the belief that they are safe with little or no toxic effects, they are linked to the chemophobic notion that "chemicals" equals "bad", are associated with a healthy life style and are "friendly with nature". Among the chronic diseases that affect the world population, cancer is one of the main causes of morbidity and mortality. It has been shown that various plants have a broad spectrum of biological anti-inflammatory, antioxidant and anticarcinogenic activities. Therefore, the investigation of anticarcinogenic properties of plants or their derivatives as a source of therapeutic or preventive agents is of high relevance. Essential oils are natural complex mixtures composed of secondary metabolites derived from leaves, bark or fruits that may exert antioxidant, antimicrobial, antifungal, antiviral, and anticancer activities. Several mechanisms have been proposed to underlie their anticancer properties such as antioxidant, antimutagenic and antiproliferative effects, enhancement of immune function and surveillance and modification of host microbiome. Eugenia uniflora L. (Myrtaceae), known in Uruguay as Pitanga, is a shrub widely distributed in South America. Recent studies in human cells indicate that the essential oils of leaves of E. uniflora have antioxidant properties, antibacterial activity and lack acute toxicity in mice. Since colorectal cancer is the third most commonly diagnosed malignancy and the fourth cause of cancer related mortality worldwide and E. uniflora leaves have been empirically used to treat inflammatory and digestive diseases, we have started to undertake a comprehensive study of their potential anticarcinogenic properties.

Methodology: Under the hypothesis that E. uniflora constitutes a source of molecular agents that can prevent tumor initiation and/or progression we have determined, as a first step, the antiproliferative and potentially lethal effects of leaf essential oil. The essential oil was obtained by hydrodistillation from young leaves of tree VIII.7 located at the Experimental Station of the Faculty of Agronomy, Universidad de la República at Salto (GPS: 31°19'S; 57°41'W). The Saccahromyces cerevisiae wild strain SC7K lys was used as a eukaryotic cell model. To test the effects of the E. uniflora leaf essential oil in cell proliferation, experiments were performed to the determine the proliferation kinetics of cell populations treated with different concentrations of the essential oil and compared with the control. Cell proliferation was determined by counting the number of cells as a function of time using optical microscopy. Also, cell viability was assessed using trypan blue exclusion test at each time point. To quantitatively characterize the effect on cell proliferation the following kinetics parameters were calculated according to the logistic model of population growth: doubling time, maximum growth rate time, number of stationary phase cells and lag phase time. To study the lethal effects, clonogenic survival tests were carried out. The survival probability as a function of the leaf essential oil concentration was determined.



Results and Discussion: Through the analysis of the proliferation kinetics a concentration dependent decrease in cell proliferation was observed (Fig. 1). Therefore, the essential oil extracted from E. uniflora leaves showed an antiproliferative effect in yeast cells. The determination of cell viability by trypan blue exclusion test at each time point during the proliferation study showed that all treated cells were viable. Furthermore, cell viability remained unchanged even after 24 h of exposure to the highest concentration of essential oil tested. This result shows the absence of immediate cytolytic effect of the essential oil of E. uniflora leaves. However, long term cytotoxicity cannot be discarded. Interestingly, in the clonogenic assay, an inhibition of the clonogenic capacity was observed and the decrease of the survival fraction was a function of essential oil concentration (Fig. 2). These **results** suggest that the essential oil of E. uniflora leaves may induce a delay in the progression of the cell cycle that could account for the modifications observed in cell proliferation kinetics. The clonogenic **results** also suggest that although cells remained viable after exposure to the highest concentrations of essential oil tested for more than 24 h, the treated cells were not able to resume proliferation leading to lethality. Cell cycle studies are under way to further study these effects.



Future Directions: As a second step in our study of the anticarcinogenic properties of essential oil of E. uniflora leaves, we are studying in human colon cell lines whether the essential oil of E. uniflora has antioxidant and pro-oxidant effects at the cellular level and if the inflammatory response has an immune-modulating effect. Preliminary results are being obtained to elucidate if this natural product may be a source of cancer therapeutic agents or become itself a useful phytochemical in chemoprevention or adyuvant chemotherapy.

Detection of immunogenic cell death markers in wild-type and EGFR-mutated lung adenocarcinoma cell lines exposed to chemotherapeutic agents

Dolores Aguilar-Cazares, Rodolfo Chavez-Dominguez, Heriberto Prado-Garcia, Alfonso Salgado-Aguayo, Lorenzo Islas-Vazquez, Jose Sullivan Lopez-Gonzalez

Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
doloresaguilarcazares@yahoo.com.mx; rodolfo.peacewalker@gmail.com; hpradog@yahoo.com; salgadoa@ymail.com; lorenzo.islasv@gmail.com; slopezgonzales@yahoo.com

Keywords: Calreticulin, Cisplatin, tyrosine kinase inhibitors, HMGB1, Immunogenic cell death, ATP release, EGFR mutations

Background: Lung cancer is the leading cause of cancer-related mortalities worldwide. Adenocarcinoma is the most frequent histological type and patients with this type of cancer are treated with platinum-based doublet chemotherapy. Currently, several studies have reported that patients whose tumors harbor EGFR mutations must be treated with Tyrosine Kinase Inhibitors (TKI). Compared to patients with wild-type EGFR, those treated with TKIs have a better progression-free survival. Reports indicate that Erlotinib, a reversible TKI induces in cancer sensitive cell lines, cell cycle arrest and apoptosis. In contrast, cisplatin (CDDP) induces in EGFR wild-type cell lines, cell death mediated by apoptosis. Recently, several studies indicate that some cytotoxic drugs elicit a particular kind of cell death, called immunogenic cell death (ICD). During ICD, a spatial-temporal expression of three critical molecules, known as the hallmarks of ICD, are emitted. These molecules participate as endogenous adjuvants to induce the antitumor immune response. In stages of ICD, previous to phosphatidyl-serine externalization, calreticulin is exposed on cell membrane acting as an "eat me" signal. Subsequently, during membrane blebbing formation and secondary necrosis, intracellular ATP is released to the extracellular space, where acts as "find me" signal. At the end of the ICD process, the nuclear protein HMGB1 is released for attraction and maturation of immature DCs.

Even it is known that Erlotinib and CDDP induce cell death in several cancer cell lines, no previous reports exist to indicate whether these cytotoxic drugs promote, in EGFR-mutated and EGFR-wild-type lung adenocarcinoma cell lines, the ICD.

Material and methods: Lung adenocarcinoma cell lines HCC827 and HCC4006 with EGFR sensitive mutations to Erlotinib and the A549 (EGFR wild-type) were obtained

from the ATCC. Cells cultures were treated with serial dilutions of Erlotinib (Sequoin Research Products, UK) or CDDP (Sigma-Aldrich, San Louis, MO). The HCC827 and HCC4006 cell lines were treated for 48h; meanwhile, the A549 cell line was treated for 24h, and in each case the IC50 was determined. For studying of the ICD induction, cytotoxic drugs concentration inducing 80% of inhibition, was added to cell cultures. The cytotoxic effect was measured using MTT assay. Percentages of necrotic and apoptotic cells were quantified using the annexin-V/propidium iodide assay and cytometric analysis; to evaluate whether apoptosis is caspase-3 dependent a fluorometric assay was used. As ICD markers, the intracellular ADP/ATP ratio was quantified by luminometry, the extracellular HMGB1 was quantified by ELISA, and the membrane-exposed calreticulin was detected by flow cytometry and validated by confocal microscopy.

Results: In the HCC827 cell line, Erlotinib induced apoptosis in 20% of the cell population. In the HCC4006 cell line, the TKI induced apoptosis in only 10% of cell population. In any case, the apoptosis was dependent of caspase-3 activation. Respect to ICD markers, the HCC827 cell line increased twice the ADP/ATP ratio and also the HMGB1 released. In this cell line, no membrane exposition of calreticulin at initial phases of apoptosis was detected. In the HCC4006 cell line, TKI induced an increase of two-folds the ADP/ATP ratio; however, no changes in the remaining ICD markers were detected. In contrast, in the A549 cell line, CDDP induced apoptosis in more than 60% of cell population; this cell death was dependent of caspase-3 activation. The ADP/ATP ratio increased two-folds, meanwhile the increase of HMGB1 in culture supernatants was 14-folds. Membrane-exposed calreticulin was detected as spots.

Conclusions: In the EGFR-mutated cell lines, cell death induced by Erlotinib was not immunogenic. According to the ICD markers studied, CDDP induced in the A549 cell line immunogenic cell death. Studies are required to analyze whether the ICD markers expressed in apoptotic cell or apoptotic bodies derived from the A-549 cell line, are associated to the phagocytic cells uptake.

Mesenchymal stroma cells derived from primary breast tumors have immunoregulatory properties that can favor tumor development

Elizabeth Tellez Rivera[1], Diana Reyez Hernandez[1], Alvaro Diaz Rodriguez[1], Gina Stella Garcia Romo[1,2], Alexander Pedroza Gonzalez[1,2]

[1]Unidad de Morfología y Funsión, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla, Mexico
[2]Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla de Baz, Mexico
liz.b.223@hotmail.com; dianareyezhernandez@comunidad.unam.mx; dark.razorblade681@gmail.com; garciaromogina@gmail.com; alexander_pg@hotmail.com

Keywords: breast cancer, GM-CSF, Mesenchymal stroma cells, ICOS-L, immunoregulatory molecules

Breast cancer is the most prevalent carcinoma in women worldwide. Given the current limitations in treatments the use of immunotherapy is an attractive alternative. However, a better understanding of immune regulation within the site of injury is needed to guarantee therapeutic success. The dynamic interactions of cells that form part of the tumor microenvironment can determine the type of immune response produced. Mesenchymal stroma cells (MSCs) constitute an important component of tumor stroma and their presence favors tumor development in animal models. However, there is very scarce information about their contribution to the development of human tumors. Therefore, in the present work, we analyzed the presence and functional characteristic of MSCs isolated from primary breast tumors of patients with breast cancer. Using flow cytometric analysis we found that MSCs isolated from early breast tumor were CD45-CD24-CD44+CD90+CD73+CD105+ and they expressed the regulatory molecules ICOS-L and PDL-1. In response to the inflammatory cytokines IL-1β and TNFα, tumor derived MSCs produce the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF), but not thymic stromal lymphopoietin (TSLP). It was observed that the expression of ICOS-L was slightly increased by the inflammatory stimuli, while no significant changes were observed for PD-L1.These observations suggest that MSCs derived from primary breast tumors can play an important role in the regulation of the local immune response through the expression of the molecules PD-L1 and ICOS-L and the production of GM-CSF causing an immunosuppression microenvironment and probably the induction of Th2 type responses that favors tumor growth.

Analysis of immunosuppressive capability of mesenchymal stromal cells from patients with cervical cancer against NK cells

Ana G. Serrato López[1,2], Víctor Adrián Cortés Morales[1,2], Alberto Monroy-Garcia[1], Erika Hernandez-Esteves[1,2], Hector Mayani[1], Teresa Apresa-García[1], Leticia Rocha-Zavaleta[2], Juan José Montesinos[1]

[1]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
ana-serrato@outlook.com; v.adrian.cortes@gmail.com; albertomon@yahoo.com; erwgocgl@hotmail.com; hmayaniv@prodigy.net.mx; teresa_t_a@yahoo.com.mx; lrochaz@biomedicas.unam.mx; montesinosster@gmail.com

Keywords: Immunomodulation, Mesenchymal Stem Cells, Mesenchymal Stromal Cells, NK cells, cervical cancer

Background: Mesenchymal stromal cells (MSCs) from bone marrow (BM) have been described as a population with immunosuppressive capability, which interact with immune system cells, including Natural Killer cells (NK) and modify their response. Natural Killer cells (NK) are a kind of lymphocytes which participate in defense upon viral infections and against tumor cells. NK cells perform their function through cytokine secretion such as IFNγ, TNFα and GM-CSF, and they also have cytotoxic activity.

Cervical Cancer (CeCa) in Mexico is the second cause of death due to oncologic disease in women. Our investigation group has demonstrated that MSCs are cells which constitute tumor microenvironment in CeCa tumors and favor growth of malignant cells by decreasing T-lymphocytes cytotoxical activity against these cells.

Although immunosuppressing effects of BM-MSCs cells over NK cells are well described, it is unknown how MCSs coming from a tumor microenvironment interact over NK cells. This interaction might have important repercussions in the development of CeCa.

The objective of the present study is to determine immunosuppressive capability of MSCs from tumors of patients with CeCa against NK cells.

Methods: MSCs were obtained from cryopreserved samples, previously characterized from BM samples, healthy cervix (HC), cervical epidermoid carcinoma (CeEC), and cervical adenocarcinoma (CeAd).

To obtain NK cells, blood samples from healthy donors were used, and mononuclear cells were separated by gradient density. CD56+ cells were separated by positive selection using CD56+ pearls (Miltenyi Biotec).

To evaluate NK cell proliferation, they were dyed with CFSE (50 μM) and seeded in co-culture with MSCs from the various sources for 5 days. They were stimulated with 20 ng/ml of IL-2 to induce proliferation. After this period, the non-adherent fraction was harvested and analyzed.

To evaluate production IFNγ by NK cells, NK cells were seeded in co-culture with MSCs from the various sources for 5 days in the presence of 20 ng/ml of IL-2. 24 hours before their lecture, 1ng/ml of IL-12 and 10 ng/ml of IL-18 were added to stimulate IFNγ production. They were then incubated with Monensin for 6 h, and stained for flow cytometry assessment to detect intracellular IFNγ and extracellular CD56.

To evaluate NK cells cytotoxicity, NK cells were seeded in co-culture with MSCs from the various sources for 5 days in the presence of 20 ng/ml of IL-2. After this period, non-adherent fraction was harvested and seeded in different proportions (1:1, 2:1 and 4:1; effector: target), along with cells from K562 line as targets. They were incubated for 4 h and a flow cytrometric staining was performed for CD56, AnnexinV and 7-AAD. For the analysis, CD56 population was considered.

Result: Co-culture from different sources of MSCs increased NK lymphocyte proliferation, at least 5% (HC-MSCs) and up to 11% (CeAd-MSCs).

IFNγ-producing NK cells population was increased in co-cultures with HC-MSCs and CeCa-MSCs, while with BM-MSCs, no significant differences were observed in comparison with control. According to our results, MSCs from cervix have the capability to increase IFNγ-producing NK cells and therefore promote the generation of a pro-inflammatory environment.

It was found that when NK cells were in co-culture with BM-, HC- and CeAd-MSCs, their cytotoxic activity was partially reduced, but when NK cells were in co-culture with CeEC-MSCs, an increase in such activity was observed. These may be indicative of MSCs populations with distinct immunomodulating properties, depending on the type of tumor.

Conclusion: MSCs from cervical cancer might perform a polarizing effect of NK cells to a cytokine-producing population and decrease their cytotoxic activity. These results may have implications in tumor growth and development.

Soluble factors from breast cancer tumor cells induce immune cells activation, promoting invasion of non-aggressive tumor cells

Angélica Aponte-López[1,2], Ezequiel Moisés Fuentes-Pananá[2], Samira Muñoz Cruz[3]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Hospital Infantil de México Federico Gómez, Mexico City, Mexico
[3]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
angibel06@hotmail.com; empanana@yahoo.com; mcsamira@yahoo.com.mx

Keywords: Mast Cells, breast cancer, degranulation, Innate immunity cells, Tumor agressiveness

Introduction: Breast cancer is a major world health problem. In Mexico, it is the most frequent neoplasia for the 20 years and older population, representing 18.7% of all malignant tumors. It has been observed that immune cells and associated inflammation are central drivers of tumor maintenance and progression. Macrophages, neutrophils and mast cells are some of the innate immune cells found in the stroma of breast tumors that modulate the tumor behavior. Particularly, mast cells are distinguished by having a wide array of preformed bioactive molecules stored in intracellular cytoplasmic granules, which are immediately released upon encountering the appropriate stimuli (mechanism know as degranulation). Moreover, mast cells are also capable to induce de novo synthesis of different inflammatory mediators, such as lipid compounds and cytokines. However, the participation of mast cells in breast cancer is not well known.

Goal: To evaluate mast cells activation by conditioned media (CM) from breast tumor cells with different aggressive potentials and the role of activated mast cells in the promotion of the tumor aggressive features.

Methods: HMC1 and LAD-2 mast cell lines were stimulated with CMs from luminal MCF-7 and triple negative MDA-MB-231 breast cancer lines (non-aggressive and aggressive, respectively). Mast cell activation was measured by a degranulation assay and IL-8, IL-5 and VEGF expression by qPCR. Invasion assays were performed using stimulated mast cells as chemotactic signal.

Results: We observed LAMP-1 and LAMP-2 surface deposition supporting mast cell activation by tumor cells. Similar levels of degranulation were observed with both CMs. On the contrary, IL-8, IL-5 and VEGF expression levels were higher in mast cells stimulated with CM from MDA-MB-231 cells, the most aggressive cell line. Likewise, activated mast cells induced invasion of MCF-7 cells, although this effect was more prominent for mast cells stimulated with CM from MDA-MB-231.

Conclusions: Secreted components by breast tumor cells are able to activate mast cell degranulation and de novo transcription. IL-8, IL-5 and VEGF expression was higher in mast cells stimulated with CM from highly aggressive MDA-MB-231 breast tumor cells. These activated mast cells also exhibited an increased capacity to promote invasion of no-aggressive tumor cells.

Role for mif in colorectal cancer development in mice C57BL/6

Imelda Juarez Avelar, Martha Ferral, Miriam Rodriguez-Sosa

Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico
imelda_juarez@yahoo.com; marthaferral17unam@gmail.com; rodriguezm@unam.mx

Keywords: colorectal cancer, C57BL/6 Mice, murine model, MIF, AOM-DSS

Colorectal cancer (CRC) is a major public health problem, it ranks 3rd in incidence and 4th in mortality among all types of cancer. Recently, an important participation of the Macrophage migration Inhibitor Factor (MIF) as a promoter of tumor growth has been suggested. Inhibition of MIF reduces the number and size of tumors in tumor cell implanting CRC murine models. In contrast, CRC patients with high levels of MIF in connective tissue had a better prognosis (survival greater than 5 years), than patients with reduced levels. This controversy has raised the need to determine the participation of MIF in the development of CRC. Therefore, we established a chemically-induced CRC model; six- to eight-week-old male C57BL/6 mice received a single injection of Azoxymethane (AOM, 10 mg / Kg) via i.p., followed by 3 cycles of 2% Dextran sodium sulphate (DSS) diluted in drinking water. After the induction, the weight of the mice, the clinical signs, and mortality were determined weekly; also, the levels of pro and anti-inflammatory cytokines in serum were quantified by ELISA. At day 63 post-induction the mice were euthanized under CO2/O2 excess atmosphere and the colon excised; length of the colon, number of tumors and degree of dysplasia were determined by visual inspection and histopathological analysis. Our results show that at day 63, mice with CRC presented no significant differences in the serum levels of IL-1b, TNF-a, IFN-γ and IL-4, but significantly higher levels of MIF compared with healthy mice. Induced mice showed a decrease in colon length, and developed an average of 12 tumors, 66.7% of these tumors were less than 1 mm in diameter and 13.3% exceeded 2 mm. The tumor load per mouse was calculated as the sum of all intestinal tumors volume. Histology showed juvenile polyps with abundant inflammatory infiltrate; rupture, invasion and destruction of the intestinal mucosa, as well as high-grade dysplasia. These results demonstrate the feasibility of the chemically induced CRC model in C57BL/6 mice, and suggest a role for MIF in the tumor development and establishment. Currently we have obtained a strain of MIF Knockout mice in C57BL/6 background for future studies to establish more accurately the role for MIF in Colorectal Cancer.

Decreased gene expression profiling of cell surface receptors related to NK cells in breast cancer tissue resistant to antineoplastic treatment

Mariel Garcia Chagollan[1], Irma Edith Carranza Torres[2], Pilar Carranza Rosales[2], María Guadalupe Martínez Silva[2], Humberto Ramírez Montoya[2], Adriana Aguilar Lemarroy[2], Luis Felipe Jave Suárez[2]

[1]University of Guadalajara, Guadalajara, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
maye_999@hotmail.com; mitzba@hotmail.com; carranza60@yahoo.com.mx; lupita694@yahoo.com; ramirez@oncologia.org.mx; adry.aguilar.lemarroy@gmail.com; lfjave@yahoo.com

Keywords: NK cells, breast cancer, NK cell receptors, Chemotherapeutic treatment, Tumor infiltrating lymphocytes (TILs)

Introduction: Chemotherapeutic scheme based on taxane and anthracycline is widely accepted as treatment in breast cancer. However, multi-drug resistance continues as a clinical challenge. As a component of the innate immune system, the cytotoxic function of NK cells has an important role in the elimination of tumor cells, including breast cancer cells. Nowadays, the role of NK cells in the resistance to systemic therapy in breast cancer remains unclear.

Objective: To evaluate the gene expression profile of human NK cells in breast cancer tissue resistant to treatment by taxanes-anthracyclines

Materials and Methods: Biopsies from tumor tissue were obtained from breast cancer patients without prior treatment as well as samples of breast tissue from women with fibroadenomas. Histopathological analysis and in vitro exposure to antineoplastic was done. Alamar Blue assay and lactate dehydrogenase release were performed for quantitative analysis of tumor viability. Gene profile expression from tumor tissue without prior exposure to therapeutic drugs was analyzed by microarray platform (Roche-NimbleGen) and verified by qPCR.

Results: A significant gene expression decrease of cell surface receptors related to NK cells was observed in tumor tissue resistant to antineoplastic treatment compared to tumor tissue that was sensitive to the treatment.

Conclusions: A decrease of NK cells infiltration in tumor tissue might be a predictive marker for failure of chemotherapeutic treatment in breast cancer.

A recombinant strain of Newcastle disease virus shows antitumor and immunostimulatory activity in vitro

Oscar A Ortega-Rivera[1], Jose L Quintanar[1], Susana Del Toro-Arreola[2], Angel G Alpuche-Solis[3], Mayra J Esparza-Araiza[3], Eva Salinas[1]

[1]Autonomous University of Aguascalientes, Aguascalientes, Mexico
[2]University of Guadalajara, Guadalajara, Mexico
[3]Institute for Scientific and Technological Research, San Luis Potosí, Mexico
eaoscarortega@gmail.com; jlquinta@correo.uaa.mx; susana@cucs.udg.mx; alpuche@ipicyt.edu.mx; mayra.esparza@ipicyt.edu.mx; emsalin@correo.uaa.mx

Keywords: Newcastle disease virus, antitumor activity, immunostimulatory activity, Genotype V, Strain P05

According to the World Health Organization, cancer is a term that groups a broad set of diseases that can affect any part of the body. It is caused by transformation of normal cells to malignant ones, which behave unusual and grow beyond their usual boundaries. It is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015. Conventional chemo and radiotherapy have many adverse effects and fail to cure many types of cancer in humans, thus alternative therapies to treat cancer patients have become more popular since late last century. Among these therapies the use of oncolytic viruses, which have tropism to malignant cells but not to normal ones, has been gaining field during the last decades. Oncolysis induced by these viruses is mostly an immunogenic type of cancer cell death that includes immunogenic apoptosis, necrosis and autophagic cell death. Additionally, these viruses have a strong immunostimulatory activity. An example of these viruses is the Newcastle disease virus (NDV), which belongs to the Paramyxoviridae family and infects practically all bird species. NDV has already been used for the treatment of cancer patients, due to three key factors: i) its replication in preferentially tumor cells, ii) its efficiency killing tumor cells and, iii) its limited toxicity towards normal cells. NDV is known to induce a strong immune response by stimulating the production of certain cytokines, such as different interferons (IFN-α / IFN-β) and tumor necrosis factors (TNF–α and TRAIL), which lead to activation of Natural Killer (NK) cells, macrophages and tumor-specific T cells. In addition, it is important to note that the antitumor and immunostimulant effect of NDV is directly linked to the strain used in therapy and that the combination of different NDV strains avoids the limiting effect of neutralizing antibodies during virotherapy Thus, in order to increase NDV-therapy

efficiency, and make safer, cheaper and more accessible therapy against cancer, the objective of our study was to determine if a recombinant version of the NDV, generated from a Mexican genotype V strain (rNDV-P05), has antitumor and immunostimulatory capacity in vitro.

NDV was clarified from a commercial vaccine by ultracentrifugation in 20% sucrose cushion. The virus was suspended in PBS and its concentration and activity were determined by hemagglutination assay. To determine optimal pH for viral activity, ultracentrifuged virus was suspended in PBS solutions with different pH (3, 5, 7.4 and 9). To evaluate optimal temperature, the virus was diluted in PBS pH 7.4 and incubated at 22, 37, 42, 60 and 80˚C for 2h. For antitumor activity determination, NDV was incubated at 0 (negative control), 10, and 50HU (Hemagglutination Units) with HeLa, HCC1954, HL-60, HepG2, A549 cancer cell lines or with normal cells (PBMC) for 24 and 48h. After that, cytoplasm nucleosomes (apoptosis indicator) were measured by ELISA and expressed as Enrichment Factor (EF), which represent the quotient of treated against negative control. For immunostimulatory activity, PBMC were isolated from healthy donors using LymphoprepTM and cultured 24h with 0 (negative control), 10, 20HU of NDV or LPS (5μg/ml). Supernatants of each group were collected and analyzed by ELISA for IFN-α, IFN-γ, TRAIL, and TNF-α production. Early cytokine expression was demonstrated culturing PBMC for 4h with 0 (negative control), 20HU of NDV or LPS (5μg/ml) and later total RNA was extracted, retrotranscripted and relative expression to β-actin determined by qPCR.

Hemagglutination titers of rNDV changed according to pH, with the lowest value observed at pH 3 (1:32; $P < 0.001$), and one titer decreased at pH 5 and pH 9 (1:64; $P < 0.001$) compared to pH 7.4 (1:128). On the other hand, temperatures higher than 42˚C totally abolished hemagglutinating activity of the virus. Antitumor activity of rNDV P05 was determined as the capacity of the virus to induce apoptosis on different tumor cell lines. HeLa, HCC1954, HL-60, and HepG2 cell lines showed sensitivity to rNDV-P05, mostly in a dose and time dependent way (at least $P < 0.05$ vs control). A549 cells and PBMC were not susceptible to apoptosis induction at any time or with any used concentration of rNDV-P05 ($P>0.05$ vs control). Stimulation of PBMC with both doses (10 and 20HU) of rNDV P05 induced the secretion of high amounts of TNF α ($P<0.001$), IFN α ($P<0.01$), TRAIL ($P<0.01$), and IFN γ ($P<0.01$) after 24h of incubation, compared to negative control. LPS treated PBMC only increased significantly the secretion of TNF α ($P<0.001$) and IFN γ ($P<0.05$) against control group. PBMC treated with 20HU of virus increased the early expression (4h) of TNF α 3.06 fold ($P<0.01$), TRAIL 8.64 fold ($P<0.01$), IFN α 10.80 fold ($P<0.01$), and IFN γ 11.41 fold ($P<0.05$), compared to unstimulated PBMC. At this time, LPS treated PBMC only increased significantly the expression of TRAIL compared to control group ($P<0.05$).

This study shows for the first time that a genotype V NDV has antitumor and immunostimulatory activities. Because rNDV-P05 showed promising antitumor activity and was able to stimulate the expression of four key antitumor cytokines (TNF-α, IFN-α, TRAIL and IFN-γ), the present study paves the way for its use in an alternate application of different strains during virotherapy.

Mesenchymal stromal cells derived from cervical cancer induce the expression of CD73 on cervical cancer cells through the production of TGF-β and IL-10

Luis Roberto Avila Ibarra[1], Maria de Lourdes Mora Garcia[1], Jorge Hernandez Montes[1], Rosario Garcia Rocha[2], Alberto Monroy Garcia[2]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
luischaper@hotmail.com; lulumora@yahoo.com; jhzms@yahoo.com; rgrbiologia@hotmail.com; albertomon@yahoo.com

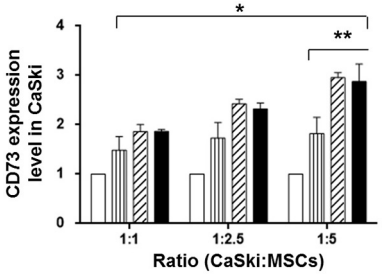
Keywords: Adenosine, Mesenchymal Stromal Cells, IL-10, TGF-b, CD73

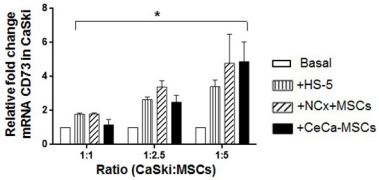
Introduction: Mesenchymal stromal cells (MSCs) are important components in the tumor microenvironment and participate together with tumor cells in the suppression of the antitumor immune response through the production of immunosuppressive factors such as TGF-β and IL-10. It is also known that these factors induce the expression of 5'-nucleotidase (CD73) in various cell types, whose functional activity is associated with the production of adenosine (Ado), an immunosuppressive nucleoside.

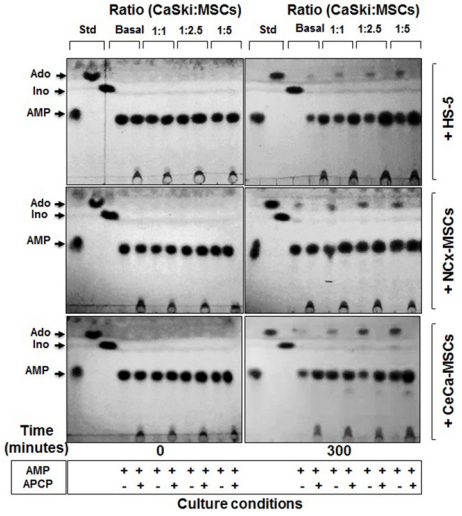
Objective: To analyze whether MSCs derived from tumors of patients with cervical cancer (MSCs-CeCa) induce the expression of CD73 in CaCu cells through the production of TGF-β and IL-10.

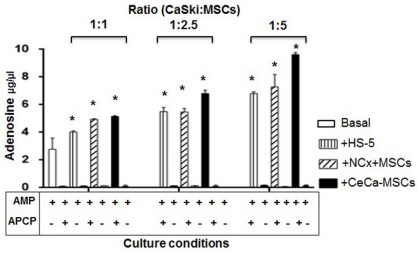
Materials and Methods: MSCs derived from either normal cervix (MSCs-NCx) or cervical cáncer (MSCs-CeCa) were co-cultured during 96 hours with CaSki (HPV-16+) cervical cancer cell line at ratios 1:1, 2.5:1 and 5:1 (CaSki:MSCs) using transwell inserts and in the presence or absence anti-TGF-β and anti-IL-10 neutralizing antibodies. The content of TGF-β and IL-10 in the supernatants was determined by ELISA assays. The expression of CD73 and its functional activity on CaSki cells was determined by culturing the cells in the presence of the substrate AMP.

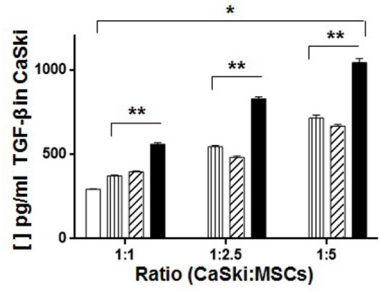
Result: The contents of TGF-β and IL-10 in the supernatants as well as the expression of CD73 on CaSki cells increased in a ratio dependent manner (CaSki:MSCs). Interestingly the addition of either anti-TGF-β or anti-IL-10 or both neutralizing anti-

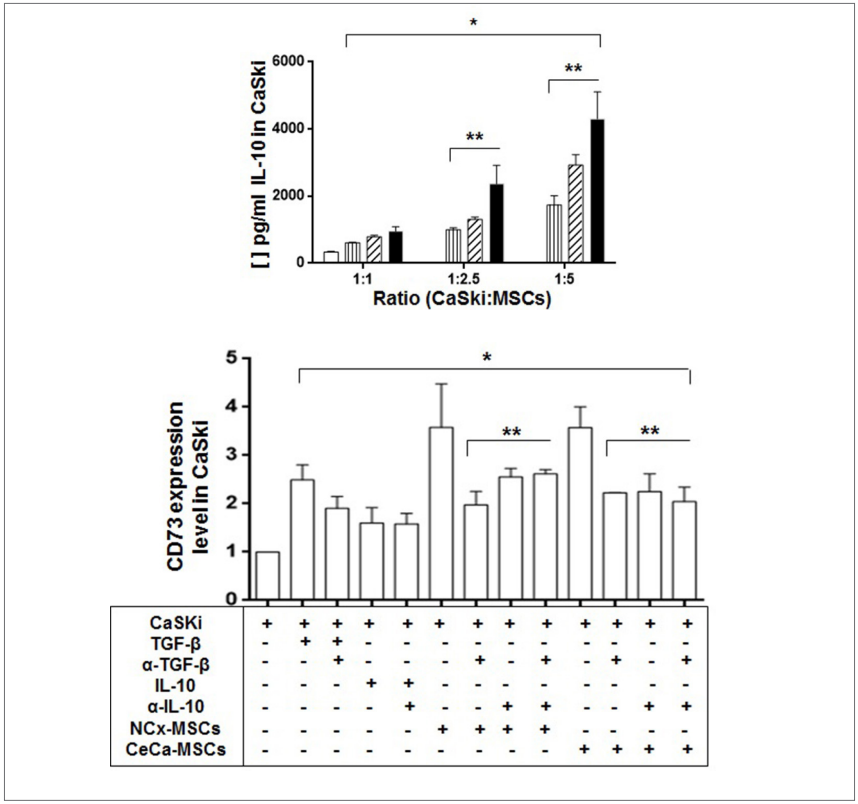

CD73 expression level in CaSki
Ratio (CaSki:MSCs)
1:1
1:2.5
1:5
*
**


Relative fold change mRNA CD73 in CaSki
Ratio (CaSki:MSCs)
Basal
+HS-5
+NCx+MSCs
+CeCa-MSCs


Ratio (CaSki:MSCs)
Std
Basal
1:1
1:2.5
1:5
Ado
Ino
AMP
+ HS-5
+ NCx-MSCs
+ CeCa-MSCs
Time (minutes)
0
300
AMP
APCP
Culture conditions


Ratio (CaSki:MSCs)
1:1
1:2.5
1:5
Adenosine µg/µl
Basal
+HS-5
+NCx+MSCs
+CeCa-MSCs
AMP
APCP
Culture conditions


[ ] pg/ml TGF-β in CaSki
Ratio (CaSki:MSCs)
1:1
1:2.5
1:5
*
**



bodies in the cell co-cultures reversed about 50% the expression of CD73 on CaSki cells and its capability to generate Ado.

Conclusions: These results suggest that MSCs present in the tumor microenvironment play an important role to induce the expression of CD73 on tumor cells through the production of TGF-β and IL-10 and maintain the local suppressive condition.

Increased plasmatic detection of the ectonucleotidases CD39 and CD73 during cervical cancer progression is correlated with the generation of increased amounts of adenosine

Ricardo Muñoz Godinez[1,2], Maria de Lourdes Mora Garcia[1], Jorge Hernandez Montes[1], Alberto Monroy García[1,2]

[1]*Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico*
[2]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
ricc_master@hotmail.com; lulumora@yahoo.com; jhzms@yahoo.com; albertomon@yahoo.com;

Keywords: Plasma, CD73, CD39, Ceca, ADO

Introduction: Cervical cancer (CeCa) is a cellular alteration that originates near the squamous junction of the cervix and is attributable to infection by human papillomavirus (HPV) in about 99.9% of cases (1). HPV infection has a long-standing natural history that begins with changes in the cervical epithelium that is known as cervical intraepithelial neoplasia (CIN) and are classified as CIN I, II, and III, according to the amount of epithelium that has been invaded that gradually transforms into invasive carcinoma (2). On the other hand, one of the factors that influence the growth of malignant tumors is the ability to overcome or avoid host defense mechanisms, in which many signaling pathways are involved during progression of cancer. Recently, the purinergic pathway has been proposed as an important modulator of the tumor growth, immunosuppression and metastasis. In this pathway, nucleotides ATP / ADP and AMP are hydrolyzed by ectonucleotidases CD39 and CD73 respectively to generate adenosine (ADO). ADO has an important role for the development of cancer, it is also established that the extracellular ADO among other molecules regulates the immune system since the increase in ADO concentrations has an immunosuppressive effect on the cells of the immune response, which favors the development of cancer (3–5). More recently, it has been demonstrated in vitro that cancer cells secrete a soluble form of CD39 and CD73 that are involved in the production of extracellular ADO, suggesting that these soluble ectonucleotidases could be traveling through tissues to inhibit the activity of immune cells (6). Therefore, it is important to know the presence of these ectonucleotidases and their capability to generar ADO during the development of the CeCa.

Objective: To determine the presence and functional activity of the CD39 and CD73 ectonucleotidases in plasma of healthy donors and patients with different stages of the CeCa (HPV infection, low and high grade of CIN and CeCa).

Methodology: Plasma samples were obtained from 15 normal donors without HPV infection, 35 from patients with HPV infection, 30 from patients with precursor lesions (high and low grade CIN) and 19 from patients with epidermoid CeCa. The detection and quantification of CD39 and CD73 ectonucleotidases in plasma samples was carried out by means of ELISA tests and using standard curves of the recombinant CD39 and CD73 proteins. The catalytic activity of the CD39 and CD73 ectonucleotidases of the plasma samples was carried out by incubation of plasma aliquots with 5mM of adenine nucleotides ATP/ADP and AMP, respectively, in the presence or absence of POM-1(a selective CD39 inhibitor) and APCP (a selective CD73 inhibitor). After 72 hours, the generation of adenosine was evidenced by thin layer chromatography and quantified by ultra efficient liquid chromatography (UPLC).

Result: Increased plasma concentration of the CD39 and CD73 ectonucleotidases was found in relation to the degree of disease progression. In fact, plasma concentration of CD39 in the samples of patients with CIN-III and CaCU was 2.3 and 2.2 times; while that of the CD73 was 1.5 and 2.6 times than that detected in plasmas derived from normal donors and patients with HPV infection.

Interestingly the capability to generate adenosine through the catalytic activity of CD39 and CD73 was strongly correlated with the presence of these ectonucleotidases in plasma samples. Samples from patients with CIN I, CIN III and CaCU generated 3–5, 14–20 and more than 30 times of adenosine in relation to that generated by samples derived from normal donors and patients with HPV infection. The addition of POM-1 or APCP almost completely inhibited the catalytic activity of CD39 and CD73, respectively.

Conclusion: The increased plasmatic concentration of the active ectonucleotidases CD39 and CD73 during cervical cancer progression could play an important immunossuppressive role through the generation of adenosine.

REFERENCES

1. Torre, L. Bray, F. Siegel, R. Ferlay, J. Lortet-tieulent, J. and Jemal, A. Global cancer Statistics, 2012. CA: A Cancer jorurnal for clinicians. 2015. 65; 87-108.
2. Bosch, F. Lorincz, A. Munoz, N. Meijer, C. and Shah, K. The causal relation between human papillomavirus and cervical cancer. J Clinic Phatol. 2002. 55(4): 244-265.
3. Young, A. Mittal, D. Stagg, J and Smyth, M. Targeting cancer-derived adenosine new therapeutic approaches. American Associations for Cancer Research. 2014. 879:888.

4. Schuler, P. Westerkamp, A. Kansy, B. Bruderek, K. Dissmann, P. Dumitru, C. Lang, S. Jackson, E. Brandau, S. Adenosine metabolism of human mesenchymal stromal cells isolatedfrom patients with head and neck squamous cell carcinoma. Immunobiology (2016).
5. Antonioli, L. Pacher, P. Vizi, E and Haskó, G. CD39 and CD73 in immunity and inflammation. Trends Mol Med. 2013. 19(6):355-367.
6. Zhang, B. Oppotunities and challenges for anti-CD73 cancer therapy. Future Medicine. 2012. 4(9); 861-865.

Tumor aggressiveness is promoted through cell to cell communication

Yessica Sánchez-Ponce[1,2], Ezequiel Moisés Fuentes-Pananá[1], Nancy Adriana Espinoza-Sánchez[1,3], Rosana Pelayo[4], Jennifer Enciso[4,5,6], Eduardo Vadillo[7], Alberto Monroy-Garcia[8], JC Balandrán[7]

[1]*Hospital Infantil de México Federico Gómez, Mexico City, Mexico*
[2]*Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico*
[3]*Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico*
[4]*Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico*
[5]*Center for Complexity Sciences, National Autonomous University of Mexico, Mexico City, Mexico*
[6]*Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico*
[7]*Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico*
[8]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
yes103_neutron@hotmail.com; empanana@yahoo.com; latmw_yo@hotmail.com; rosanapelayo@gmail.com; jenny_gr8@msn.com; evadillo@hotmail.com; albertomon@yahoo.com; jcbalandran@gmail.com

Keywords: Communication, breast cancer, invasion, aggressiveness, pro-inflammatory cytokines.

Introduction: Breast cancer (BrC) is the most common cancer affecting women worldwide. BrC is highly heterogeneous, comprising several diseases with different clinical outcomes. This is importantly due to a high inter-tumor heterogeneity in which different cells confer different proliferative, survival, metabolic and invasive capacities. The factors contributing to tumoral heterogeneity, the dynamics of interactions between different cellular types within the tumor microenvironment, as well as their contribution to tumor aggressiveness are still poorly understood.

Objective: To determine if breast tumor cells with different molecular classification communicates with each other and whether characteristics of tumor aggressiveness can be promoted through this communication.

Material and Methods: Non-aggressive luminal BrC cell lines (NA-BrC) were cultured with conditioned media (CM) from highly aggressive triple negative BrC (HA-BrC) cell lines and markers of epithelial to mesenchymal transition, stemness and chemokine receptors were analyzed by immunocytochemistry and flow cytometry and invasiveness in transwell assays was assessed using different chemoattractants. Bioinformatical analysis was performed based on array expression of 84 cancer stem cell related genes.

Results: We observed lateral transmission of aggressive characteristics from aggressive tumor cells to non-aggressive cells, consisting of loss of epithelial characteristics and gain of a stemness phenotype. Aggressive cells have high basal levels of proinflammatory mediators: G-CSF, GM-CSF, MCP-1, IL-8 and metalloproteases 1 and 2. Aggressive-induced cells showed an increase in the expression of the receptors for CXCL12, CXCR4 and CXCR7, as well as of invasiveness in response to CXCL12. Expression arrays identified a signaling footprint characterized by the activation of NFκB but without evidence of the involvement of TGF-β on induced-aggressive cells. In vitro tests corroborated that TGF-β does not participate in the induction of aggression, even though it is the best characterized molecule that mediates epithelial to mesenchymal transition, while supporting the participation of NFkB.

Conclusions: We demonstrate the communication between tumor cells with different molecular classification and aggressive potential, a biological mechanism that could influence the dynamics of the intra-tumoral population by promoting the appearance of clones with new functions. Understanding these interactions will provide better targets for diagnosis, prognosis and treatment.

IL-10, TGF-β induce the expression of CD73 in membrane and the release of soluble CD73 in tumor cells of cervical cancer

Gabriela Molina Castillo[1], Jorge Hernández Montes[1], Alberto Monroy-Garcia[2], Benny Weiss Steider[1], María de Lourdes Mora García[1]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
mus_h_u25@hotmail.com; jhzms@yahoo.com; albertomon@yahoo.com; bennyweiss@hotmail.com; lulumora@yahoo.com

Keywords: Adenosine, IL-10, TGF-β, CD73 expression, sCD73

Introduction: Adenosine plays an important role in the suppression of the antitumor immune response, in addition to promoting growth and metastasis in several tumor types. This is generated mainly through the enzymatic activity of CD73 on the nucleotide AMP. CD73 is found anchored in the cell membrane or in soluble form (sCD73). On the other hand, it has been proposed that pro-inflamatory and anti-inflamatory cytokines, among others, can modulate the expression of CD73 in different cell types. In the particular case of cervical cancer (CeCa) it has been reported that the presence of the anti-inflammatory cytokines TGF-β and IL-10 are increased in tissues and in plasma of patients with advanced CeCa. In this study we evaluated the effect of TGF-β and IL-10 in the induction of CD73 on cell membranes as well as in the release of sCD73 of CeCa tumor cells.

Materials and Methods: Cervical cancer cells CaSki (HPV-16+) were cultured in the presence of 20ng / mL of each of the following cytokines: TGF-β, and IL-10. After 72 hours, the expression of CD73 on membrane was determined by flow cytometry, and the release of sCD73 in the supernatant of cell cultures by ELISA. The catalytic activity of this enzyme was analyzed by culturing the cells, or supernatants, with 5mM of AMP for 24, 48 and 72 hours, both in the presence and absence of 5mM of APCP (specific inhibitor for CD73). The adenosine produced was evidenced by thin layer chromatography and quantified by high performance liquid chromatography (UPLC).

Results: Tumor cells cultured after 72hrs in the presence of TGF-β showed a significant increase in the expression of CD73 in the cell membrane. However, the treatment of

the tumor cells with both IL-10 and TGF-β increased by at least twice the amount of sCD73 with respect to the basal production of this ectonucleotidase. On the other hand, the increase in the expression of CD73 in the membrane or sCD73 was correlated with higher ability to hydrolyze AMP and generate adenosine. In all cases, the addition of APCP significantly reduced the hydrolysis of AMP.

Conclusions: The induction of the CD73 expression on cell membrane as well as in a soluble manner in CeCa tumor cells treated with TGF-β or IL-10, suggests that the presence of these cytokines during the development of CeCa may be important to maintain an immunosuppressive state and favor tumor progression through the generation of adenosine.

Study of the participation of mif in the development of intestinal microbiota during the genesis of murine CRC

Octavio Hernandez Rosas, Imelda Juarez Avelar, Oscar Illescas, Thalia Pacheco-Fernández, Miriam Rodríguez Sosa

Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla de Baz, Mexico
hpapayo1@hotmail.com; imelda_juarez@yahoo.com; oscarillescas@comunidad.unam.mx; silaht16@gmail.com; rodriguezm@campus.iztacala.unam.mxn

Keywords: mouse models, colorectal cancer (CRC), dss colitis, MIF, microbiome modification

Chronic inflammation of the intestine is an important risk factor for the development of colorectal cancer (CRC). In addition, dysbiosis (alteration of normal microbiota) has been related to the development of various pathologies including CRC, revealing large differences between microbial populations of healthy individuals and patients. Macrophage migration inhibitory factor (MIF), is an inflammatory cytokine that is over-expressed in large number of cancer types, including CRC. It has been associated with modulation of the immune response and tolerance directed towards various microorganisms.

Given the importance of MIF in the regulation of inflammation and immune response against bacteria, by inducing the expression of TLR4, we decided to evaluate the effect of this cytokine on the composition of intestinal microbiota, and if this composition plays a role in the development of CRC. We developed a CRC model in pathogen-free BALB/c mice that were treated with antibiotics to decrease their microbial populations. Subsequently, mice received microbiota transfers from healthy non-treated WT or MIF -/- mice. CRC was induced by administering azoxymethane (AOM) intraperitoneally (10mg/Kg), and 3 cycles of 2% DSS diluted in drinking water. Two experiments were carried out: The first culminated on day 40 and the second on day 68 post-induction with AOM. The weight of the mice was measured throughout the experiments, while the length of the colon and the number of tumors were determined at the end of the experiments. MIF levels were determined in situ and in blood serum by ELISA, and transcripts for pro and anti-inflammatory cytokines by RT-PCR. The degree of dysplasia was evaluated by histological analysis, and bacterial microbiota component populations were determined by 16S sequencing.

The results show that at day 40, similarly developed intestinal adenomatous polyps appeared in both WT and MIF-/- microbiota receptors. However, on day 68 the mice receiving microbiota from MIF -/- donors developed significantly less tumors than WT donor microbiota receptors, although larger. These results suggest that microbiota, whose conformation has been influenced by MIF, plays an important role in the development of colorectal cancer. Future experiments will identify the microbial populations present, and determine the influence of MIF on its conformation and the malignancy of tumors developed.

STAT1 signaling protects mice from colorectal cancer development and modulates the recruitment of inflammatory monocytes and granulocytes

Sonia Leon-Cabrera, Armando Vazquez Sandoval, Emmanuel Molina Guzman, Luis Ignacio Terrazas Valdés

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
soleon81@gmail.com; armiin.vazsan@gmail.com; dayaknegro@gmail.com; literrazas@unam.mx

Keywords: Colitis, Monocytes, STAT1, Colon Cancer, JAK/STAT signaling pathway

Colitis-associated colon cancer (CAC) is a tumor that develops under chronic inflammatory conditions present in the intestinal tissue, is different from sporadic cancer and is one of the most important causes of mortality in patients with inflammatory bowel diseases (Lasry, Zinger et al. 2016; Romano, F et al. 2016). Signal transducer and activator of transcription 1 (STAT1), is a main signaling pathway that controls critical events in intestinal immune function in response to Type I or Type II interferons (IFNs) (Slattery, Lundgreen et al. 2013) (Ramana, Gil et al. 2002). However, the role of STAT1 during inflammation-associated cancer is not clearly understood. In the present study, we evaluated the role of STAT1 in CAC development. Following CAC induction with azoxymethane (AOM) and DSS exposure, STAT1 knockout (STAT1-/-) mice displayed an accelerate appearance of inflammation and tumor formation as early as 20 days after CAC-induction that was coupled with an increase in the carcinogenesis-related enzymes Cox2 and nuclear β-catenin at the colon compared to wild-type mice (WT). Interestingly, STAT1-/- animals increased epithelial cell-proliferation in early steps of tumor progression and decreased apoptosis at the colon. TNF-α and iNOS expression were augmented in the intestinal microenvironment of STAT1-/- animals but most prominently a higher IL-17A production was detected compared to WT mice. Furthermore, monocyte recruitment was altered under STAT1 deficiency with reduced monocytic CD11b+Ly6C+Ly6G- cells accumulation whereas granulocytic CD11b+Ly6C-Ly6G+ cells were importantly increased at systemic level, which was associated with enhanced growth and progression of colorectal cancer. Finally, the anti-apoptotic protein Bcl2 was over-expressed in STAT1-/- colons supporting the idea that STAT1 is crucially involved in the initial steps of tumor development modulating immune responses and controlling mechanism like apoptosis and proliferation.

Collectively, these findings suggest a role for STAT1 as a tumor suppressor molecule in inflammation-associated carcinogenesis.

REFERENCES

Lasry, A., A. Zinger, et al. (2016). "Inflammatory networks underlying colorectal cancer." Nat Immunol 17(3): 230-240.

Ramana, C. V., M. P. Gil, et al. (2002). "Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling." Trends Immunol 23(2): 96-101.

Romano, M., D. E. F. F, et al. (2016). "From Inflammation to Cancer in Inflammatory Bowel Disease: Molecular Perspectives." Anticancer Res 36(4): 1447-1460.

Slattery, M. L., A. Lundgreen, et al. (2013). "JAK/STAT/SOCS-signaling pathway and colon and rectal cancer." Mol Carcinog 52(2): 155-166.

Regulation of immunophenotype modulation of monocytes-macrophages from M1 to M2 by prostate cancer cell culture supernatant via the transcription factor STAT3

Alejandro Bravo-Cuellar[1], Georgina Hernandez-Flores[1], Pablo Cesar Ortiz-Lazareno[1], Raúl Solis-Martínez[1], Luis Felipe Jave Suares[1], Adriana Aguilar-Lemarroy[1], Alejandro Bravo-Hernández[2]

[1]Centro de Investigación Biomédica de Occidente (CIBO), Guadalajara, Mexico
[2]Universidad Autónoma de Guadalajara, Zapopan, Mexico
abravoc@prodigy.net.mx; gina.geodic1967@gmail.com; pablolazareno@gmail.com; raulsolis12357@gmail.com; lfjave@gmail.com; adry.aguilar.lemarroy@googlemail.com; alexbra.bravo@gmail.com

Keywords: prostate cancer, M2, stat3, M1, Stattic™

Background: Prostate cancer is an important public health problem because it is the most frequently diagnosed type of cancer and is the second most common cause of cancer-related death in men worldwide. The transcription factor STAT3 has a prominent innate immunity effect on cancer progression. We determined the regulation of STAT3 in the immunophenotype modulation of macrophages from M1 to M2 induced by the cell culture supernatant of the prostate cancer line PC3.

Methods: Monocytes-macrophages from healthy donors were cultured in the supernatant of PC3 cells, membrane proteins, and intracytoplasmic and phosphorylated STAT3 were measured using flow cytometry, while cytokines and growth factors were studied using luminescence. Cytotoxicity and nitric oxide were evaluated via colorimetric assays.

Results: The supernatant of prostate tumor PC3 cells effectively induced macrophages toward an M2 profile, and the expression of phosphorylated STAT3 in the monocytes-macrophages notably increased, and mainly related to IL-10. In the group of monocytes-macrophages treated with a STAT3 inhibitor, the macrophages were induced towards an M1 phenotype.
Conclusions: In this study, we showed that the secretion profile of PC3 prostate cancer cells induces a change in macrophage phenotype from M1 to M2, and that phenomenon is related to phosphorylation of the transcription factor STAT3 and IL-10.

Pentoxifylline induces apoptosis in human retinoblastoma cancer Y79 cells and increased effects to Carboplatin-induced apoptosis. Caspase-8 activity and NF-kB participation

Georgina Hernandez-Flores[1], Alejandro Bravo-Cuellar[1], Pablo Cesar Ortiz-Lazareno[1], Claudia Carolina Cruz-Galvez[1], Adriana del Carmen Aguilar-Lemarroy[1], Luis Felipe Jave-Suarez[1], Alejandro Bravo-Hernandez[2]

[1]*Centro de Investigación Biomédica de Occidente (CIBO), Guadalajara, Mexico*
[2]*Universidad Autónoma de Guadalajara, Zapopan, Mexico*
gina.geodic1967@gmail.com; abravocster@gmail.com; pablolazareno@gmail.com; carolina_clau1227@yahoo.com.mx; adry.aguilar.lemarroy@googlemail.com; lfjave@gmail.com; alexbra.bravo@gamil.com

Keywords: Apoptosis, Carboplatin, Pentoxifylline, IκBα, Y79 cells

Background: Retinoblastoma (Rb) is the most common primary intraocular malignancy of infancy and early childhood. To date, conventional treatments for Rb have comprised enucleation, cryotherapy, radiotherapy or chemotherapy. Carboplatin (CARB) is a DNA damage-inducing agent that is widely used for Rb treatment. However, unfortunately, this drug also activates the Nuclear Factor Kappa Beta (NF-kB) pathway, leading to the activation of cell survival the mechanisms. On the other hand, it has been reported that in vivo and in vitro in several tumor types, Pentoxifylline (PTX) increases the toxic action of antitumor drugs such as Adriamycin, MG132, and Cisplatin and NF-kB inhibition. This study investigates the possible effects of PTX on Y79 human Rb cells.

Methods: An in-vitro experimental study was conducted with Rb Y79 cells that were incubated in presence of PTX or CARB or its combination, and thereafter the following parameters were evaluated cell viability, cell cycle, apoptosis, mitochondrial membrane potential loss, cytochrome c release, caspase-3, -8, and -9 activities, IkBa phosphorylation and expression of pro- and anti-apoptotic BAD, BAK, BAX, and CASPASES -3, -8, and-9 genes.

Results: Our results surprisingly showed that both drugs significantly affected the viability of the Rb cell line Y79 in a time- and dose-dependent manner. The PTX + CARB combination showed the important, utmost apoptosis, cell-cycle arrest, caspase-8

activity, and NF-κB inhibition. We also observed, upregulation of BAD, BAK, BAX, and CASPASES 3, -8, and -9 genes in favor of the apoptotic pathway.

Conclusions: PTX induces apoptosis per se and sensitizes toward carboplatin-induced apoptosis by a balance in favor of proapoptotic signals, caspase-8 activity, and NF-kB inhibition. These observations support the idea that PTX represents a potential candidate for targeted antitumor therapy for Rb and are agreeing with the concept of rational chemotherapy with molecular basis.

Peripheral blood T cells in pathologic complete response after neoadjuvant chemotherapy for luminal locally advanced breast cancer

M. Carmen Arango-Prado[1], María Elena Faxas-García[1], Ramón Ropero-Toirac[1], Pilar Soto-Pardeiro[1], Carlos Agustín Villegas Valverde[1], Inés Pérez-Barojo[1], María Caridad Rubio-Hernández[1], Iraida Sánchez-Varela[1], Rolando Camacho-Rodríguez[1], Zahili Gutiérrez-Aleaga[1], Janet Lamadrid-García[1], Gretell León-Cruz[1], Elías Gracia-Medina[1], Cristina Rodríguez-Padilla[2]

[1]Instituto Nacional De Oncología Y Radiobiología (INOR), La Habana, Cuba
[2]Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico
carmenap@infomed.sld.cu; mefaxas@infomed.sld.cu; ropero@infomed.sld.cu; pilarsoto@infomed.sld.cu; carlosvillega@infomed.sld.cu; ines@infomed.sld.cu; rubio@infomed.sld.cu; iraida@infomed.sld.cu; rolandocamacho@infomed.sld.cu; zaily@infomed.sld.cu; lmadrid@infomed.sld.cu; gretel.leon@infomed.sld.cu; elias@infomed.sld.cu; crrodrig@ccr.dsi.uani.mx

Keywords: Neoadjuvant Therapy, NK cells, breast cancer, Treg, Pathology response

Introduction: Breast cancer is one of the most frequent malignant tumor of women all over the world. The stage III, or locally advanced breast cancer, is a heterogeneous disease and usually requires neoadjuvant chemotherapy (NAC). The evaluation of pathological response following NAC is an essential independent prognostic factor and, in particular, the pathologic complete response (pCR) is a good indicator of survival. On the contrary, the no pathologic complete response (no pCR) could be associated to unfavorable clinical response. This tumor can be distinguished into subgroups based on the gene expression profiles and molecular classification. This classification handle by the scientific community allows to go deeper into the molecular characteristics of this particular tumor and provides the pathologic complete response (pCR) the more specific treatment to the patients. The subtypes of breast cancers according to their gene signature are as follows: Luminal A; Luminal B (HER2/neu negative or HER2/ neu positive); HER2 overexpression and Basal like (triple negative). Luminal A and Luminal B HER2/neu negative, include 70% of invasive breast cancers, which are estrogen receptor/ progesterone receptor positive, HER2 negative. The difference between luminal B subtype and luminal A, is a higher proliferation index rate expressed by Ki-67 in the first one. Previous results have shown that the immune system may acts in favor to host-protective or to tumor-promoting ways. However, our understanding of the complex interplay between breast cancer cells and immune system cells is incomplete. Results from several studies have demonstrated that in breast cancer

patients there are modifications in CD8+ cytotoxic T lymphocyte (CTL), CD4+ helper T (TH) cells, CD4+/CD25+/FOXP3+ T regulatory (Treg) cells and in natural killer (NK) cells present in tumor microenvironment, lymph nodes and in peripheral blood (PB). All these changes could be related with prognostic factors already established such as the pathological response after NAC. The aim of this study is to evaluate the possible association of pCR with the phenotype markers expressing by CTLs, T reg and NK cells of peripheral blood

Materials and Methods: Was included 166 women with locally advanced breast cancer and range of age, 20–65 years old. The patients were grouped according to current TNM classification after admission at the Mastology Service of National Institute of Oncology and Radiobiology, Havana, Cuba. We selected only patients with a histologically diagnosis of invasive ductal carcinoma, specifically molecular subtypes Luminal A and Luminal B HER2/neu negative (by pre- therapy tru-cut biopsy) and eligible for neoadjuvant chemotherapy (taxane-based regimens) followed by surgical treatment. This study was conducted in compliance with the Declaration of Helsinki, approved by the Ethics Committee and written informed consent was obtained from all patients according to the protocol approved. The immunological phenotype of PB was evaluated before initiation of the neoadjuvant chemotherapy by Flow Cytometric Analysis according to conventional procedures. The immunological phenotypes were determined using several monoclonal antibodies (MoAbs) (Dako, Denmark): anti-human CD3/PerCP Clone UCHT1, anti-human CD4/RPE Clone MT310, anti-human CD8/FITC, Clone DK25, anti-human CD16 (Fc Gamma Receptor III)/ FITC Clone DJ130c and anti-human CD56/RPE Clone C5.9. The phenotype CD4+/CD25+/FOXP3+ of T reg cells was evaluated by Human Treg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD4 PE-Cy5/CD25 PE) from BioLegend. The cells were assessing with Gallios™ Flow Cytometer (Beckman Coulter), 100000 events each sample were considered. The results were expressed in cell absolute numbers.

Tumor sample was available after local surgery for Pathological Response evaluation according to Miller-Payne Grading System.

Result: The results of the cells determinations before chemotherapy treatment were evaluated and, after surgery, the specific pathological Response was determined. In patients with no-pCR the mean values of NK cells (CD3-/CD16+/CD56+) and CTL (CD3+/CD4-/CD8+) were statistical significantly lower (p=0.06 and p=0.08, respectively), than in pCR patients and healthy donors (control group). Meanwhile, Treg (CD4+/ CD25+/ FOXP3+), cells were statistical significant higher (p=0.002) in no-pCR. All the results were analyzed by ANOVA (one way) and Kruskal-Wallis tests.

There were an statistical significantly association between pCR or no-pCR Treg levels (Chi-square=16,25, p=0,000); NK (Chi-square=57,9, p=0,000) and CTL (Chi-square=15,9, p=0,000). The Bivariate Logistic Regression showed that when the Treg cells values are high, upon a 95% Confidence Interval for Mean control values (>IC 95%), the probability of no pCR is also increased (OR=20.925; p=0.000). On the contrary, when NK cells and CTL values are low (< IC 95%), increase the probability of no pCR (OR=51.911; p=0.000 and OR=6.304; p=0.000) respectively. These three determinations are independent variables.

Discussion: Local advanced breast cancer is a disease traditionally treated by chemotherapy. Not all patients achieve good response and although the molecular classification helps in treatment decisions, this disease is also related with the competence of the immune system.

Our results show that an increase of Treg cells, decreased NK cells and CTL before chemotherapy treatment, have a higher probability of not have a complete pathological response. This association suggests that an immune deregulation is present in these patients, with suppression of the anti-tumoral activity of CTL and NK. On the other hand, patients with normal values of immune cells get greater benefit with chemotherapy with pathological complete response.

The immunomodulatory effects of chemotherapy are better if the Treg cells are in such normal values that are not able to induce immunosuppression. An immune response could also be achieved by chemotherapy-induced cell death, mainly in local advance breast cancer to control the possible remaining tumors cells and distant metastasis.

Tregs cells, as part of the immune response, probably interfere with the NK cells and CTL normal function, and thus they are crucial in breast cancer disease due to the relationship between suppression and anti-tumor immune effector T cell response.

Conclusions: Pretreatment low values of CTL and NK cells, and a high number of Treg, cells are related with no pathologic complete response (no-pCR) after neoadjuvant chemotherapy. In this group of patients, a deregulation of the immune system would compromise the induced anti-tumoral response to neodayuvant chemotherapy. It is possible to suggest that the peripheral lymphocytes (NK, CTL, T reg) would be useful to evaluate as a response biomarker in local advanced breast cancer patients.

p16 overexpression and high levels of Treg cells in smokers young patients with oropharyngeal cancer

María Elena Faxas-García[1], María del Carmen Arango-Prado[1], Yanisei Duarte-Pérez[1], Carlos Agustín Villegas Valverde[1], Darien Hernández[1], Elisabeth Caraballoso[1], Julio Jiménez[1], Cristina Rodríguez-Padilla[2]

[1]Instituto Nacional De Oncología Y Radiobiología (INOR), La Habana, Cuba
[2]Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico
mefaxas@infomed.sld.cu; carmenap@infomed.sld.cu; yanisei@infomed.sld.cu; carlosvillega@infomed.sld.cu; darien@infomed.sld.cu; elisa@infomed.sld.cu; julioj@infomed.sld.cu; crrodrig@ccr.dsi.uanl.mx

Keywords: Cancer, NK cells, HPV, Treg, oropharingeal

Introduction: The incidence of oropharyngeal carcinoma has been growing, especially in younger aged groups. The International Agency for Research against Cancer (IARC) recognized human papillomavirus (HPV), especially HPV16, besides smoking and alcohol consumption, as an important risk factor for oropharyngeal squamous cell carcinoma (OPSCC). HPV positive patients have better prognosis than HPV negative ones which is an important observation of relevance that allows a more specific treatment. p16 (CDKN2A) expression is a useful surrogate marker related with tumor HPV status in oropharyngeal cancer, and these results were validated in the retrospective analysis of RTOG0129. The HPV infection is controlled by the host immune system and if the infection is not cleared, a tumor development is possible, mainly when the host has unfavorable modifications of the immune system. Patients with locally advanced OPSCC can still be treated with a curative intention but different factors should be considered.

We determined the number of lymphocytes subpopulations in peripheral blood as well as in tumor samples in young smoker patients with OPSCC p16 positive.

Material and Methods: All patients included attended the Head and Neck service of the Institute of Oncology, Havana, from 2011–2016. Twenty two young patients (14 males and 8 females), (median age: 33 years; range 20–35 years), with locally advanced OPSCC were studied. The primary tumor was base of tongue in 14, and tonsil in 8 patients. The research was conducted according to the Declaration of Helsinki, approved by the Ethics Committee and written informed consent was obtained from all patients included in the final protocol. The first step was an individual interview to know risk associated factors such as the frequency of tobacco and alcohol consumption. All laboratory tests

were done before therapy. The immunological phenotypes were determined using the following monoclonal antibodies (MoAbs) (Dako, Denmark): anti-human CD3/PerCP Clone UCHT1, anti-human CD4/RPE Clone MT310, anti-human CD8/FITC, Clone DK25, anti-human CD16 (Fc Gamma Receptor III)/ FITC Clone DJ130c and anti-human CD56/RPE Clone C5.9. The phenotype CD4+/CD25+/FOXP3+ of T reg cells was evaluated by Human T reg Flow™ Kit (FOXP3 Alexa Fluor® 488/CD4 PE-Cy5/CD25 PE) from BioLegend. The cells were assessing with Gallios™ Flow Cytometer (Beckman Coulter), 10000 events each sample were considered. The results were expressed in cell absolute numbers. A control group of twenty healthy individuals was included. White blood count (WBC) was detected using the routine laboratory procedure. The mean levels of the immunological parameters measured in the peripheral blood were compared between the groups of patients and controls.

Tumor samples were obtained by surgery and conventional diagnostic procedures were performed.

Immunohistochemical staining for lymphocyte determination in tissue samples were done using DAKO labeled avidin-biotin-peroxidase method (LSAB+) and 3,3′-diaminobenzidine (DAB) as the chromogen. The Mabs included were to determine CD3, CD4, CD8, FoxP3 and CD20 positive cells (DAKO).

All patients were subjected to immunochemistry assays for p16. It was performed on 4 µm paraffin-embedded tissue sections using p16INK4 mouse anti-human antibody (clon E6H4) with DAB detection kit. It was considered positive p16 when more of the 70 % of tumor cells were staining.

Result: Twenty patients were smokers and eighteen out of twenty are current smoking lasting for five years. Fourteen patients were alcohol consumers at least twice a week. After the immunohistochemistry evaluation, seventeen tumors (80%) were p16-positive.The tissue samples from 15/17 patients showed an infiltration by intra tumoral FOXP3 T reg cells. In peripheral blood, comparing with the healthy control, the patients had different results. Those difference were statistical significant. There were elevated levels of T regs cells (mediums values=85.671 cells/ml, $p>0.05$), low values of CD8 cytotoxic T lymphocyte (CTL) (mediums values=423.678 cells/ml, $p>0.05$), and also low NK values (mediums values=148.853 cells/ml, $p>0.05$). There were no associations of the different lymphocyte subsets with clinical and tumor variables.

Discussion: The persistence of HPV infection is essential in the progression of HPV-related cancers. The virus itself creates different mechanisms to escape host immune surveillance control, responsible to clear the infection. During the initial steps of

tumor development, the known etiological factors, such as smoking, alcohol, and HPV infection, the progression of the disease is associated with failures of the host immune system. Tobacco use is capable to suppress immune function, facilitating infection persistence. Indeed, it helps to prevent a normal immune response to eliminate the virus related cancer cells because of the release of several elements such as nicotine, hydroquinone and carbon monoxide, toxic for cells, increasing carcinogenesis and immunosuppresion. Moreover, tobacco induces DNA breaks in human cells and increases the carcinogenic potential of HPV.

The innate immune system plays a role in anti-tumor immunity. NK cells are part of the innate immune defense against virus and cancer. The p16-positive smoker patients had lower values of NK cells and it means that the lack of these cells interfere with the necessary effector functions with reduced cytolytic activity. Usually, decreased amounts of these cells are associated with an increased risk of recurrence, and poor outcome. On the other hand, the low values of CD8+ cells emphasize the unfavorable modifications of the immune system of these patients. The changes observed in the T cell compartment may be also related to the fact that smoking suppresses certain Th1 responses, facilitates Th2 inflammation, and reduces the phagocytic and killing functions of macrophages and also the survival of these cells.

This study identified FOXP3+ Treg cells in tissue samples and high values in peripheral blood. This is a contradictory result, because elevated levels of FOXP3+Treg are related with an increased immune activation and also as a significant predictor of "cause-specific mortality" in patients with OPSCC.

Nowadays, more individualized approach based on specific tumor or patient characteristics help to differentiate patients who need an aggressive treatment and those who will not, avoiding unnecessary side effects and expensive costs, However, it should be emphasized, that within the HPV or p16-positive cases, several subsets of patients with different prognosis should be identified. The favorable impact on the prognosis of HPV-positive patients is not the only parameter, especially in countries where the patients have the tobacco habit.

Autophagy regulates the secretion of Macrophage Migration Inhibitory Factor (MIF) from Breast Cancer Cell Lines

Paola Maycotte[1,2], Johari Salgado-Gallegos[2,3], Israel Cotzomi-Ortega[2,3], Arely Rosas-Cruz[2,4], Lourdes Millán-Pérez-Peña[3], Patricia Aguilar-Alonso[3], Miriam Rodriguez-Sosa[5], Julio Reyes-Leyva[2]

[1]Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico City, Mexico
[2]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
[3]Benemérita Universidad Autónoma de Puebla, Puebla, Mexico
[4]Universidad Nacional Autónoma de México, Mexico, Mexico
[5]Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla de Baz, Mexico
bisbenzimida@gmail.com; bisbenzimida@gmail.com; salgadoj@live.com.mx; salgadoj@live.com.mx; israelcotzomi@gmail.com; israelcotzomi@gmail.com; arelyrosascruz@gmail.com; arelyrosascruz@gmail.com; loudra50@yahoo.com.mx; agapmx@yahoo.com.mx; rodriguezm@unam.mx; julio.reyes@imss.gob.mx

Keywords: Autophagy, breast cancer, cytokine, inflammation and cancer, macrophage migration inhibitory factor (MIF)

Macrophage Migration Inhibitory Factor (MIF) is a pro-inflammatory cytokine that promotes macrophage metabolism, phagocytosis, adherence and T cell proliferation. In cancer, MIF is involved in tumor progression and anti-tumor immunity. It activates MAPKs, induces angiogenesis, promotes tumor invasion through metalloprotease secretion and it antagonizes p53. In anti-tumor immunity, MIF has a role in macrophage polarization and in the activation of myeloid-derived suppressor cells. MIF is constitutively expressed in macrophages and does not have an amino-terminal localization sequence, indicating its secretion involves an unconventional protein secretion route.

Autophagy (from greek auto, self and phagos, eat) is a protein and organelle degradation pathway in which cytoplasmic contents are engulfed in a double membrane structure named autophagosome and delivered to the lysosome for degradation. The role of autophagy in cancer is complex, acting as a tumor suppressor mechanism during carcinogenesis and as a tumor promoting pathway during tumor progression. Autophagy has recently been involved in the secretion of cytokines and mediators of invasion like IL6, IL8 and metalloproteinases from cancer cells as well as in the secretion of pro-inflammatory mediators from immune cells.
In this work we found that autophagy regulates the secretion of MIF from breast cancer cell lines. Although MIF was constitutively secreted from breast cancer cell lines of

different subtypes, particularly the ones belonging to the triple negative subtype, autophagy inhibition increased MIF release to the culture medium but only in non-invasive cell lines. Our results underscore the need to understand the mechanism of autophagy regulation of MIF secretion and the role of MIF in tumor progression, metastasis and tumoral immune response since autophagy is currently being manipulated in clinical trials for the treatment of breast and other types of cancer.

Vaccination-induced skin-resident CD8+ T cells mediate and broaden protective immunity against melanoma

Felipe Ignacio Gálvez-Cancino[1], Ernesto Lopez Cabrera[1], Evelyn Menares[1], Ximena Díaz[1], Camila Flores[1], Pablo Cáceres[1], Sofía Hidalgo[1], Ornella Chovar[1], Marcela Alcántara-Hernández[2], Vincenzo Borgna[1], Manuel Varas-Godoy[3], Flavio Andres Salazar Onfray[4,5], Juliana Idoyaga[2], Alvaro Lladser[1]

[1]Fundación Ciencia and Vida, Santiago, Chile
[2]Stanford University, Stanford, United States
[3]Facultad de Medicina, Universidad de los Andes, Santiago, Chile
[4]Instituto de Ciencias Biomédicas, Universidad de Chile, Santiago, Chile
[5]Millennium Institute on Immunology and Immunotherapy (MIII), Santiago, Chile
figalvez88@gmail.com; ernesto.lopez@ug.uchile.cl; evelyn.menares@gmail.com; ximena.diazo@usach.cl; cam.floresgonzalez@gmail.com; pablo.caceres.m@ug.uchile.cl; hidalgo.t.sofia@gmail.com; ornellachovarvera@gmail.com; marceahe@stanford.edu; vborgna@gmail.com; varas.manuel@gmail.com; fsalazaronfray@gmail.com; jidoyaga@stanford.edu; alladser@cienciavida.org

Keywords: Melanoma, Vaccination, antitumor immunity, tissue-resident memory T cells, cytotoxic CD8+ T cells

Long-lasting memory CD8+ T cells have the potential to control primary and disseminated tumors. Resident-memory CD8+ T (Trm) cells stably reside in non-lymphoid tissues and mediate superior protective immunity against pathogens. Emerging evidence indicates that Trm cells develop in human solid cancers and play a key role in controlling tumor growth. However, the specific contribution of Trm cells to antitumor immunity is incompletely understood. Moreover, vaccination strategies that efficiently establish Trm cell responses remain largely unexplored and are expected to strongly protect against tumors. Here we demonstrated that a single intradermal administration of gene- or protein-based vaccines efficiently induces specific Trm cell responses against models of tumor-specific and self-antigens, which accumulated in vaccinated and distant non-vaccinated skin. Vaccination-induced Trm cells were largely resistant to in vivo intravascular staining and antibody-dependent depletion. Intradermal, but not intraperitoneal vaccination, generated memory precursors expressing skin-homing molecules in circulation and Trm cells in skin. Interestingly, vaccination-induced Trm cell responses strongly suppressed the growth of B16F10 melanoma, independently of circulating memory CD8+ T cells, and were able to infiltrate tumors. Interestingly, Trm-mediated melanoma rejection lead to the generation of secondary cytotoxic CD8+ T cell responses against a melanoma-derived self-antigen, providing protection against

re-challenge with B16F10 cells lacking vaccine-targeted antigen. This work highlights the therapeutic potential of vaccination-induced Trm cells against skin malignancies and their ability to broaden circulating CD8+ T cell responses to potentially control antigen-loss escape mutants.

Analysis of mitochondrial content and mitochondrial membrane potential in CD8+ T cells from lung cancer patients

Rosa Sandoval-Martinez, Susana Romero-Garcia, Jose Sullivan Lopez-Gonzalez, Uriel Rumbo-Nava, Heriberto Prado-Garcia

Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
rosx_05@hotmail.com; sugar_cia@yahoo.com; slopezgonzalez@yahoo.com; urumbo@gmail.com; hpradog@yahoo.com

Keywords: Flow Cytometry, Mitochondria, lung cancer, CD8+ T cells, Immunometabolism

Lung cancer is the most common type of cancer with the highest rates of mortality worldwide. Adenocarcinoma is a subtype of non-small cell lung cancer that accounts for 40% of all cases. Malignant pleural effusion is associated with advanced stages of lung adenocarcinoma; this condition **results** from an excessive accumulation of fluid in the pleural space that it is thought to be the result of an imbalance between formation and removal of pleural fluid. Pleural effusion frequently contains tumor cells and immune cells like CD8+ T cells. In a variety of human solid cancers, CD8+ T cells play an important role in anticancer immune response, because of their ability for recognizing tumor-associated antigens presented on MHC class I molecules by tumor cells and the capacity to directly destroy these cells.

It is increasingly recognized that adequate nutrient supply and energy production are key determinants of the capacity of T cells to proliferate and mediate effector function. Naive and resting T cells make use of fatty acid oxidation and the mitochondrial tricarboxylic acid cycle (TCA), which provides reducing agents for energy production through oxidative phosphorylation (OXPHOS). Upon activation, CD8+ T cells have been described to switch their metabolism to become heavily dependent on glycolysis, even in the presence of sufficient oxygen.

CD8+ T cells shown a dramatic increase in glucose metabolism after activation. Cancer cells may inhibit T cell metabolic reprogramming to impair antitumor immunity through both direct signaling and indirect effects, altering the local composition of nutrients and waste products present in the tumor microenvironment. While the effects of glucose deprivation on glycolytic metabolism and T cell function in the tumor microenvironment have garnered much interest, the mitochondrial phenotype of T cells remains unclear.

Recently, different reports describe an important role for mitochondrial metabolism during activation of antigen-specific CD4+ and CD8+ T cells. Activation induces metabolic flux through TCA to generate citrate for lipid biosynthesis and provide electron donors for the electron transport chain (ETC). Activation of the ETC is important for signaling events during T cell activation partly mediated by reactive oxygen species (ROS), which promotes T cell expansion and cytokine production. Thus, alterations in mitochondria might cause a deficient antitumor response.

In this study we analyzed mitochondrial mass (MM) and mitochondrial membrane potential (MMP) in CD8+ T cells from pleural effusion mononuclear cells (PEMC, 9 adenocarcinomas and 1 mesothelioma) and peripheral blood mononuclear cells (PBMC, nine adenocarcinoma patients and one small cell lung cancer) from lung cancer patients. As comparison groups, we included PEMC from exudative non-malignant pleural effusion (comprising six tuberculosis, one heart failure, one hemothorax, one pneumonia and one empyema) and PBMC from healthy donors (12 volunteers).

Mononuclear cells from all groups were stimulated with anti-CD3 antibody or bead-coated with anti-CD3, anti-CD2 and anti-CD28 antibodies for 24 hours. We analyzed total CD8+ T cells in peripheral blood and pleural effusion and to examine CD8+ T cell subpopulations, we used a combination of CD45RA and CD27 markers. Mitochondrial mass (MM) was analyzed using MitoTracker Green, which is a green-fluorescent mitochondrial stain that localizes into the mitochondria regardless of mitochondrial membrane potential. Also, we evaluated mitochondrial membrane potential (MMP) using DiOC6(3) (3,3'-Dihexyloxacarbocyanine Iodide), which is a cell-permeant, green-fluorescent, lipophilic dye that is selective for the mitochondria of live cells; DAPI (4',6-diamidino-2-phenylindole) and Annexin V were used to discriminate of necrotic and apoptotic cells. MM and MMP were measured by multiparametric flow cytometry on a FACSCANTO II or a FACS ARIA II and the data were analyzed using the softwares FlowJo v9.9.4, Plestle v1.7 and SPICE v5.35.

Mitochondrial mass, measured as mean fluorescence intensity (MFI) of MitoTracker Green, was higher in pleural effusion CD8+ T cells compared to peripheral blood CD8+ T cells from lung cancer patients, healthy donors and non-malignant pleural effusion under non-stimulated conditions. We analyzed mitochondrial mass in memory (CD45RA-CD27+) and naive (CD45RA+CD27+) CD8+ T cells, only memory CD8+ T cells from lung cancer patients had increased MFI values of MitoTracker Green (Figure 1A and B). Also, our analysis showed that there were two sub-subsets based on mitochondrial content: cells with high mitochondrial mass (MMhigh) or low mitochondrial mass (MMlow). In pleural effusion from lung cancer patients, increased percentages of MMhigh cells within the naive and memory CD8+ T subsets

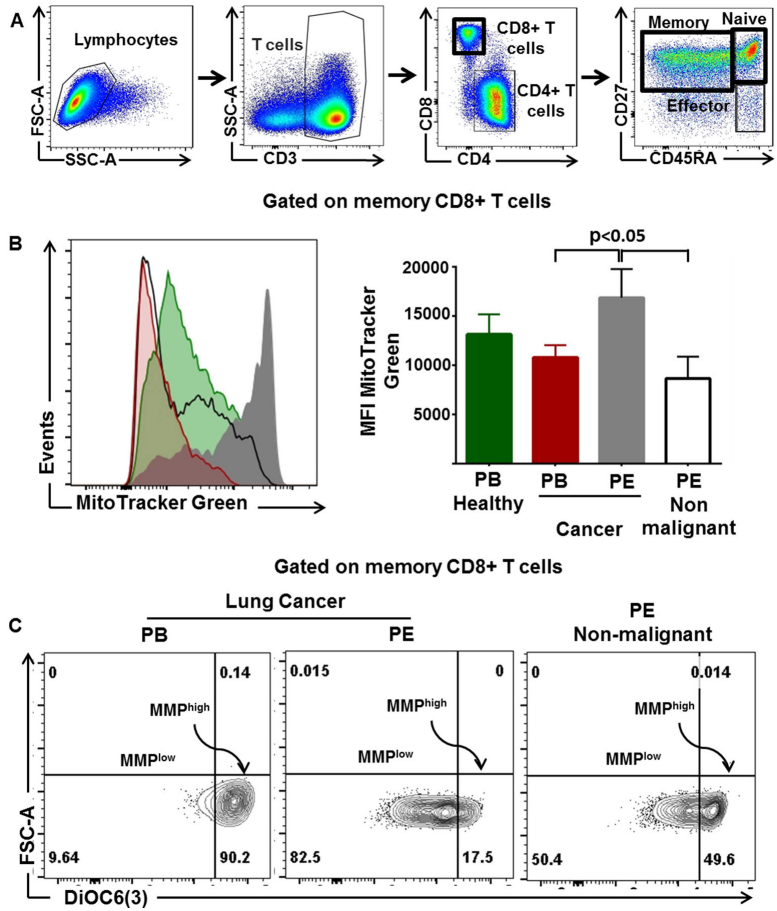


Figure 1. Pleural effusion CD8+ T cells fr om lung cancer patients show increased mitochondrial mass and mitochondrial despolarization. **A)** Representative gating strateg y for CD8+ T cells, first, singlets are identified and gated on a FSC-A vs FSC-H graph, then live cells were gated on a FSC-A vs DAPI-A graph, data not shown. The lymphocyte population was identified by forward scatter (FSC) and side scatter (SSG) T cells (CD3+) were gated and then CD4+ and CD8+ T cells were identified. Dot pot distribution of naive, memory and effector CD8+ T cells is shown. **B)** Anatysis of corresponding MFI values of Mito Tracker Green staining on memory CD8+ T cell subset. n= 10 pl ura t effusion and n=10 peripheral blood (PB) from lung cancer patients, n= 10 non-malignant pleural effusion (PE) and n=10 peripheral blood (PB) from healthy donors. **(C)** Mitochondrial membrane potential measurements were performed using DiOC6(3) on a representative sample from lung cancer patient and a non-malignant pleural effusion sample MMP^{low}= Mitochondrial membrane potential low, MMP^{high}= Mitochondrial membrane potential high.

were observed. Nevertheless, subsequent to stimulation, we did not find differences in mitochondrial mass content.
When we evaluated mitochondrial membrane potential (MMP) in CD8+ T cells from any of the groups studied, we found two subsets: cells with high mitochondrial membrane potential (MMPhigh) or low mitochondrial membrane potential (MMPlow). In pleural effusion memory CD8+ T cells from lung cancer patients, MFI values of DiOC6(3) were lower in MMPlow subset compared to the same subset from non-malignant pleural effusions (Figure 1C). We did not find differences in the MFI values from MMPhigh subsets in any of the groups analyzed.

Subsequent to stimulation with bead-coated anti-CD2, anti-CD3 and anti-CD28 antibodies, in pleural effusion memory CD8+ T cell subset from lung cancer patients MFI values of DiOC6(3) were lower in the MMPlow subset compared to the corresponding subset from non-malignant pleural effusions. MMPhigh subset in pleural effusion naive CD8+ T cells from lung cancer patients showed lower MFI values of DiOC6(3) subsequent to the stimulation with beads compared to non-stimulated conditions ($p < 0.05$). Additionally, after stimulation with anti-CD3 antibody, naive CD8+ T cells from malignant effusions showed lower MFI values of DiOC6(3) in the MMPlow subset compared to the same subset from non-malignant pleural effusion ($p < 0.05$).

These data indicate that pleural effusion CD8+ T cells showed a mitochondrial phenotype characterized by increased mitochondrial mass associated to depolarization of mitochondrial membrane potential. Thus, in the pleural effusion compartment, the tumor microenvironment seriously impairs the mitochondrial metabolism of CD8+ T cell from lung cancer, which might compromise the antitumor function of these cells.

Macrophage migration inhibitory factor is a positive modulator if macrophage-driven-immune response in colorectal tumors at early stages of cancer progression

Thalia Pacheco-Fernández[1], Oscar Illescas-Pomposo[1], Jonadab Efraín Olguin[1], María Sandra Bautista-Cuellar[1], Rogelio Hernandez-Pando[2], Imelda Juarez Avelar[1], Luis Ignacio Terrazas-Valdés[1], Miriam Rodriguez-Sosa[1]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
silaht16@gmail.com; oscarillescas@comunidad.unam.mx; efra004@gmail.com; sandy_strella15@hotmail.com; rhdezpando@hotmail.com; imelda_juarez@yahoo.com; literrazas@unam.mx; rodriguezm@unam.mx

Keywords: colorectal cancer (CRC), Tumor immune response, macrophage migration inhibitory factor (MIF);, azoxymethane-dextran sodium sulfate, MIF-knockout mice

Colorectal Cancer (CRC) is triggered by chronic inflammation (1). Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that promotes angiogenesis, metastasis, proliferation and survival in malignant cells (2). When MIF binds to CXCR2 and CXCR4 attracts macrophages and T cells, respectively (3). As MIF promotes inflammation and recruit immune cells, we investigated the role of MIF in murine model of CRC. Mif-/- and WT BALB/c mice received an intraperitoneal inyection of 12mg/kg azoxymethane and later were exposed to 3 cycles of Dextran Sodium Sulfate (DSS, 2%) in drinking water. After the first DSS cycle WT mice received a synthetic MIF's inhibitor (CPSI-156). All mice were killed 68 days after CRC induction. Greater burden of tumors were observed in both Mif-/- (25.38±1.772) and CPSI-156-treated mice (18.83±2.43) compared to CRC-WT mice (13.00±1.862). Tumors from Mif-/- mice were histologically-identified as more aggressive. Lower percentages of tumor-associated macrophages were found in both, Mif-/- (0.6577 ± 0.117) and CPSI-156-treated mice (1.2 ± 0.14) compared to CAC-WT mice (2.044±0.39). Similar percentage of T-cells was observed in all groups. RT-PCR of tumors was performed, relative expression (RE) was compared to β-actin gene. CRC-WT mice increased T-regs (foxp3 RE= 0.49), M2 (arginase-1 RE=1.53) and IL-10 (il-10 RE=0.52) and reduces expression of IL-17 (il-17 RE=3.14). Conversely, CRC-Mif-/- mice developed higher IL-17 (il-17 RE=17.15) and lower foxp3 (RE=0.32), arginase-1 (RE=1.12) and il-10 (RE=0.47) expression. In early stages of tumor development (day 34), we didn´t find differences in tumor burden among WT and Mif-/- CRC groups. We found in MIF-deficient mice lower F4/80 expression than WT mice and M1/M2 ratio. Our results

suggest that MIF regulates tumor microenvironment attracting macrophages to the tumor site and favoring M1 activation since the beginning of tumor development condition that may favor the establishment of antitumor responses at later stages.

REFERENCES

1. Rubin, D.C., A. Shaker, and M.S. Levin, Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. Front Immunol, 2012. 3: p. 107.
2. Bucala, R. and S.C. Donnelly, Macrophage migration inhibitory factor: a probable link between inflammation and cancer. Immunity, 2007. 26(3): p. 281-285.
3. TILLMANN, Sabine, et al. Arrest functions of the MIF ligand/receptor axes in atherogenesis. Frontiers in immunology, 2013, vol. 4, p. 115.

Relationship of Interleukin 33 with metastasis-supporting events in colorectal cancer patients

Glauben Tamara Landskron[1], Marjorie K De La Fuente[1], Diego Romero[2], Karen Dubois-Camacho[1], Santiago Sepulveda[2], David Díaz Jiménez[1], Rodrigo Quera[3], Daniela Simian[3], Antonella Sanguineti[4], Sebastian Lopez[4], María-Julieta González[1], Udo Kronberg[3], Hector Contreras[1], Mario Abedrapo[4], Cristina Peña[5], Guillermo Diaz-Araya[1], Juan Carlos Roa[2], Marcela A. Hermoso[1]

[1]Universidad de Chile, Santiago, Chile
[2]Pontificia Universidad Católica de Chile, Santiago, Chile
[3]Clinica Las Condes, Santiago, Chile
[4]Hospital Clínico de la Universidad de Chile, Santiago, Chile
[5]Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain
glaulandskron@ug.uchile.cl; mkdelafu@gmail.com; biobancopuc@gmail.com; karenduboisc@gmail.com; sasepulvedag@gmail.com; davordijim@gmail.com; rquera@clinicalascondes.cl; dsimian@clinicalascondes.cl; asanguineti@gmail.com; sebastianlopez@gmail.com; jgonzale@med.uchile.cl; ukronberg@clinicalascondes.cl; hcontrer@med.uchile.cl; mabedrapo@hcuch.cl; cristinapenamaroto@gmail.com; gadiaz@ciq.uchile.cl; jcroas@gmail.com; mhermoso@med.uchile.cl

Keywords: colorectal cancer (CRC), desmoplasia, epithelial mesenchymal transition, IL33, Cancer associated fibroblasts (CAF)

Colorectal cancer (CRC) is the third most frequent cancer worldwide. Tumor microenvironment (TM) is relevant in tumorigenesis and cancer progression, with cancer associated fibroblasts (CAFs) being its most abundant component. CAFs are a phenotypically heterogeneous population, and alpha-smooth muscle (alpha-SMA) is one of the most consistent markers. CAFs facilitate tumor progression by inducing angiogenesis, immune suppression and invasion by modification of the organization/composition of the extracellular matrix (i.e., desmoplasia) and/or activating epithelial - mesenchymal transition (EMT). Recently, IL33/ST2 pathway has gained huge interest as a protumor alarmin, promoting progression to metastasis mostly by inducing changes in tumor microenvironment in a variety of in vitro and animal models.

Therefore, our aim was to evaluate the role of IL33/ST2 in metastasis-related processes such as desmoplasia and EMT in CRC.

For this purpose, 41 patients were recruited from three centers (Bio-bank from Universidad de Chile, BTUCH; Coloproctology Departments from CLC and HCUCH),

mean age (SD) 66.6 (14.7), 46% women, AJCC Stage I 12%, Stage II 36%, Stage III 48%, Stage IV 0; 63.4% patients with left-sided CRC.

Total IL33 and ST2 content were analyzed in tumor and healthy tissue lysates and plasma from CRC patients by ELISA, tissue localization and distribution of both molecules in epithelia or stroma, with alpha-SMA, vimentin and E-cadherin as reference were evaluated by immunohistochemistry and quantified by Aperio Imagescope software.

In vitro experiments were conducted in primary cultures of CAFs and normal fibroblasts (NFs) isolated from tumor and healthy tissue from CRC patients. qPCR of EMT markers (E-cadherin, N-cadherin and vimentin), cell migration and proliferation analysis were performed in HT29 and HCT116 cell lines. Mann-Whitney test or t-test were performed accordingly as the quantitative data followed a normal distribution. Lineal regression and correlation coefficients were calculated between quantitative histopathological data and clinical data. A p-value lower than 0.05 was considered significant.

IL33 content in left-sided CRC is increased in those patients with lymphatic metastasis (p=0.007), and its localization in tumor epithelia is associated with abundant desmoplasia (r [IC] = 0.4 [0.01–0.7], p=0.003). Although ST2 content is similar between tumor and healthy tissue, a decreased immunoreactivity was observed in lymphatic metastasis (*p*=0.0085) and left-sided tumor stroma (p=0.0359). This decrease was associated to metastasis related factors, such as more advanced staging (r [IC] = -0.56 [-0.8 - -0.16], p=0.007), abundant desmoplasia (r [IC] = -0.52 [-0.78 - -0.11], p=0.013) and presence of tumor budding (p=0.03). Principal component and clustering analysis including stromal and epithelial IL33/ST2 and alpha-SMA immunoreactivity (with extent of desmoplasia) allowed us to distinguish clusters of low, intermediate and abundant desmoplasia.

In vitro experiments showed that recombinant human IL33 is an early transient trigger of alpha-SMA expression and stress fibers assembly in CAFs from CRC patients (p=0.01 only at 24h), and potentiating TGF-beta effect at this timepoint (IL33 + TGF-beta, p < 0.0001 at 24h), whereas TGF-beta acts as a slow steady stimulus (p=<0.0001 at 72h and 96h) stabilizing CAFs phenotype.

IL33 transcript levels from CAFs directly correlate with in CRC cell migration induced by CAFs conditioned media (R2= 0.99, p=0.0027). Also, IL33 induces a mesenchymal phenotype in HT29 cells (decrease in E-cadherin, increase in n-cadherin and vimentin mRNA, with higher cell migration). These results suggest IL33/ST2 mediates processes associated with invasion and metastasis in left-sided CRC participating in tumor microenvironment interaction, specifically between CAFs and epithelial tumor cells. Stromal and epithelial IL33/ST2 specific distribution may constitute a potential diagnostic signature for desmoplasia with beneficial prognosis assessment in CRC management.

Lung cancer immunotherapy: The Center of Molecular Immunology approach

Tania Crombet[1,2], Elia Neninger[3], Saylin Alfonso[4], Ramon Ortiz[4], Pedro Camilo Rodriguez Rodriguez[2], Maurenis Hernandez[2], Amparo Macías[2], Danay Saavedra[1,2], Zuyen Gonzalez[2], Zaima Mazorra[2]

[1]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba

[2]Center of Molecular Immunology (Cuba), Havana, Cuba

[3]Hermanos Ameijeiras Hospital, Havana, Cuba

[4]Celestino Hernandez Hospital, Villa Clara, Cuba

taniac@cim.sld.cu; nenin@infomed.sld.cu; saylin@infomed.sld.cu; ramonoc@infomed.sld.cu; camilo@cim.sld.cu; maurenis@cim.sld.cu; amparo@cim.sld.cu; danays@cim.sld.cu; zuyen@cim.sld.cu; zaima@cim.sld.cu

Keywords: Cancer Vaccines, Lung Neoplasms, racotumomab., immunotherapy of cancer, CIMAvax-EGF

Lung cancer is the first cause of death for cancer in Cuba. Unfortunately, 70 to 80 % of the patients are diagnosed at advanced stage. At the Center of Molecular Immunology (CIM), our main research focus is cancer immunotherapy. CIM pipeline includes several cancer vaccines and monoclonal antibodies against tumor specific antigens. One of the cancer vaccines, Cimavax EGF is intended to induce antibodies against EGF, which is a potent growth factor that binds EGFR with high affinity. This interaction activates a signal transduction cascade that results in cellular proliferation, angiogenesis and survival. To confirm vaccine efficacy, a Phase III randomized, placebo-control clinical trial was recently concluded. The study recruited 405 patients with advanced non-small cell lung cancer (NSCLC) patients. Vaccinated patients had a significantly larger overall survival as compared to controls, according the Harrington-Fleming estimate. Most vaccinated patients developed a high anti-EGF antibody response. Retrospective analysis of the Phase III trial pointed towards the prognostic and predictive value of the EGF concentration in patients. Advanced NSCLC patients with high serum EGF concentration after platinum chemotherapy had a worse prognosis compared to non-vaccinated patients with low EGF serum levels. In contrast, patients with high levels of EGF had a better survival if vaccinated with CIMAvax-EGF. A phase IV trial is ongoing, where Cimavax is administered at the primary care level and EGF concentration is evaluated at baseline. CIM is also developing another cancer vaccine designed as racotumomab. It is an anti-idiotypic antibody able to generate a humoral immune response against NGcGM3 ganglioside. This molecule is an attractive target for immunotherapy, since it is not present at the normal cells while is a very

potent immunosuppresor. Antibodies induced after vaccination are able to kill NGcGM3-expressing tumor cells by a complement-independent mechanism. A phase II/III multicenter, randomized, double-blind-clinical trial of racotumomab was conducted in advanced NSCLC. Racotumomab vaccine was used as switch maintenance therapy after platinum-based chemotherapy in patients with at least stable disease. Intent- to-treat analysis showed that vaccinated patients had significantly increased overall survival. Notably, the cytotoxic properties of the hyperimmune sera were associated with longer survival. A Phase III trial in ongoing where racotumomab is compared to docetaxel, as a non-inferior drug after front line chemotherapy.

CD49b+ T regulatory cells and their suppressive mechanisms differ from classical nTregs in a murine melanoma model

Pamina Contreras-Kallens[1,2], Dario Vergara[2], Karina Pino-Lagos[2]

[1]Universidad de Chile, Santiago, Chile
[2]Facultad de Medicina, Universidad de los Andes, Santiago, Chile
pamina.contreras@ug.uchile.cl; dario.vergara.slz@gmail.com; Karina.p.lagos@gmail.com

Keywords: tolerance, Foxp3, Tr1, Treg, tumor immunology

T regulatory cells (Treg) are essential for controlling T-cells activity and maintaining immunologic tolerance. However, their suppressive function can have a detrimental effect on immune responses against tumor cells. Thus, it is of great importance to study the factors altering their inhibition efficiency, therefore anti-tumor therapies can be improved. The great heterogeneity of peripheral Treg is one of the problems that have to be addressed to enhance and develop novel anti-tumoral therapies.

Within the Treg subset, a new non-classical population has been recently reported, which express high levels of CD49b molecule and, depending on their activation status, can also express the canonical Treg transcription factor Foxp3. Their therapeutic immunosuppressive effect has already been studied in murine models of diabetes and collagen-induced-arthritis, in which they display a protective effect.

Regarding their molecular phenotype and possible suppressive mechanism, in comparison to CD4+CD25+, a significantly higher percentage of CD49b+ Treg was found to express several classical Treg markers like granzyme B, IL-10, GITR, ICOS and also shows higher levels of CTLA-4. Furthermore, when injected into mice with collagen-induced arthritis, CD49b+ Treg coming from IL-10 KO mice present a less protective phenotype than those from wild-type animals, underscoring the possible involvement of this classical nTreg cytokine in their mechanism.

Nevertheless, their role in a tumor model has not been yet researched. Thus, we sought to investigate their function in a murine melanoma model. Surprisingly, we found that their presence seems to be strongly influenced by the microenvironment they encounter. Whereas in the draining lymph nodes the subset composes only around 6% of total CD4+ T cells, in the tumor, they compose almost 45% of CD4+ tumor infiltrating leukocytes (TILs), of which 15% are Foxp3+. On the other hand, Foxp3+

CD49b- composes only around 10% of CD4+ TILs, a number similar to that present in the draining lymph nodes. Furthermore, we also observed that, although in lower levels than Foxp3+ Tregs, around half of CD49b+ express Neuropilin-1, a VEGF coreceptor, which has been described to be important for the stability and function of Foxp3+ Treg suppressive phenotype and in tumor progression. However, while Foxp3+ Tregs tend to maintain their Nrp-1 levels in the tumor, compared to the ones in the draining lymph nodes, CD49b+ T cells tend to lower it, which interestingly could correlate to the increase on the levels of this molecule in Foxp3-CD49b- conventional T cells that we have seen in the tumor. Even more, we also observed that CD49b+ T cells show a differential pattern of expression of some regulatory molecules, compared to Foxp3+ Tregs: a higher median fluorescence of the exonuclease CD73 in the tumor microenvironment, which is not observed within the lymph nodes populations. Similar results were found for the gene expression of the suppressive cytokine IL-10.

Thus, our results further highlight the possible differences between the immunosuppressive mechanisms of the Tregs subsets depending on the microenvironment.

NLRP3 nuclear translocation regulates IL-4 expression in patients with cutaneous lymphoma in early stage

Luis Enrique Huanosta-Murillo[1,2], Edgar Javier Ahumada Ramos[3], Marcela Alcántara Hernández[4], Gibrán Pérez-Montesinos[5], Vadim Pérez-Koldenkova[6], Fermin Jurado-Santa Cruz[5], Antonieta Domínguez-Gómez[5], Genaro Patino-Lopez[7], Laura Bonifaz[2]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[4]Department of Microbiology and Immunology, Stanford University, Stanford, United States
[5]Centro Dermatológico Dr. Ladislao de la Pascua, Ciudad de Mexico, Mexico
[6]Laboratorio Nacional de Microscopía Avanzada, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico, Mexico
[7]Hospital Infantil de México Federico Gómez, Mexico City, Mexico
enriquehm.20@gmail.com; edgarj-tk@hotmail.com; marceahe@stanford.edu; branpm1@gmail.com; lmna.cmn.siglo.xxi@gmail.com; fermin_santac@yahoo.com.mx; dominguez035@yahoo.com.mx; gena23pat@yahoo.com; labonifaz@yahoo.com

Keywords: Lymphoma, IL-4, T cell, NLRP3, TOX

Introduction: Cutaneous T-Cell Lymphoma (CTCL) is a CD4+ T cells neoplasia. It is a slow progression neoplasia, so it is possible to study the type of T lymphocytes that participate in its control, as well as the phenotype of the cells that favor the progression of the disease. CTCL starts with plaques, which might develop into skin tumors. It has been proposed a Th1 profile during early stages, that usually changes to a Th2 profile during the progression of the neoplasia. Nevertheless, it is possible that this behavior is due to a different cytokine profile of malignant and effector CD4+ T cells present in lesional skin. To discriminate between malignant and infiltrating CD4+ T cells, several reports have used TOX expression. Recently it has been reported that TOX is overexpressed in CTCL infiltrating malignant CD4+ T cells.

Moreover, it has been reported that NLRP3 is important during the differentiation of CD4+ naïve cells towards a Th2 profile. However, expression, localization and transcriptional activity of NLRP3 in malignant CD4+ T cells is unknown. Furthermore, it is also unknown if this mechanism could be relevant in IL-4 production from malignant CD4+ T cells present in lesional skin of CTCL patients.

Objective: To characterize the cytokine profile of malignant and effector CD4+ T cells in lesional skin of CTCL patients and to elucidate the regulatory mechanism of IL-4 production in malignant CD4+ T cells of patients with CTCL in early stage.

Material and Methods: Biopsies of lesional skin of patients with CTCL in early stage were collected. By flow cytometry, immunohistochemistry and immunofluorescence, we evaluated the expression of Th1/Th2 cytokines, transcription factors and expression and localization of NLRP3 in malignant (TOX+) and no malignant (TOX-) T cells. In advanced stage skin samples, we evaluated by IHC the expression of IFNγ and IL-4 and by immunofluorescence evaluated the expression of TOX and NLRP3.

In order to know the regulatory mechanism of Il-4 production, we used the cell line HTB-176 which is obtained from lesional skin of a patient with CTCL in early stage and we evaluated the expression and location of NLRP3 by immunofluorescence. We used a NLRP3 inflammosome assembly inhibitor to evaluate the effect over IL-4 production by flow cytometry and immunofluorescence.

Result: On cultured CTCL biopsies in early stage we observed a mixed Th1/Th2 response. These mixed profile includes a normal lymphoid morphology CD4+ T cells subpopulation that produces IFNγ after activation and another population of atypical large lymphocytes are producing IL-4 even in the absence of activation. This result suggests that these atypical lymphocytes are malignant cells.

In lesional skin biopsies of patients in the different stages of the disease, we observed a drecreased in IFNγ production as the disease progresses, in the other hand, IL-4 expression increases in the tumoral stage. These results suggest a predominance of IFNγ+ cells in early stages, while IL-4+ cells are enriched in advanced stages. To elucidate in which cells IFNγ or IL-4 occurs, we examinated TOX expression in early stage of CTCL, and found the presence of TOX+ IL-4+ cells, which indicates that malignant TOX+ cells are expressing IL-4. Whereas, IFNγ+ TOX- cells discovery suggests that IFNγ is produced by infiltrating no malignant lymphocytes.

Moreover, in lesional skin biopsies from patients in plaque and tumoral stage, we found NLRP3 nuclear expression in TOX+ cells, these results indicate that NLRP3 might participate in the regulation of IL-4 production.

To elucidate if NLRP3 is involved in regulation of IL-4 production, we evaluated NLRP3 expression and localization in the cell line HTB-176. We observed cytoplasmic and

nuclear NLRP3 expression, besides the inhibition of NLRP3 inflammosome assembly, resulted in an increase in the IL-4 production. In addition we detected that the inhibitor favors the nuclear traslocation of NLRP3 receptor. These results suggest that NLRP3 has the ability to induce IL-4 expression in CTCL.

Conclusions: Our results indicate that predominance of Th2 response over Th1 in CTCL patients in advanced stages is because of an expansion of malignant CD4+ T cells (TOX+IL-4+) present in lesional skin since early stage. Additionaly our results suggest that nuclear localization of NLRP3 could be involved in the regulation of IL-4 expression in patients with CTCL in early stage. Therefore our results indicate that this mechanism might be involved in the progression of the disease and possibly participate in other types of cancer in which a Th2 response participates in the mechanism of immune evasion.

The HPV-18 E6 oncoprotein down-regulates CD40 expression

Vicente Madrid-Marina[1], Joaquín Manzo-Merino[2], Alfredo Lagunas-Martínez[1], Crysele Calderón-Corona[1], Alicia Román-González[1], Kirvis Torres-Poveda[1]

[1]Instituto Nacional de Salud Pública, Cuernavaca, Mexico
[2]Instituto Nacional de Cancerología (INCan), Tlalpan, Mexico
vmarina@insp.mx; jmanzomerino@gmail.com; alagunas@insp.mx; crys_calder@hotmail.com; alycya34@gmail.com; kjtorres@insp.mx

Keywords: HPV, oncoprotein, CD40, E6, AKNA

Human Papilloma Virus infection represents the most common sexual transmitted disease around the world with a high prevalence in both, men and women, leading to the appearance of genital verruca and in exceptional cases to cancer. Normally, HPV is limited to the site of infection and regulates a plethora of cellular elements in order to avoid the immune surveillance. During the viral infection the activation of pro-inflammatory cytokines is induced with the final aim to avoid the infection spread. It is well recognized that the HPV infection induces an anti-inflammatory state and down-regulation of CD40 surface protein, in order to progress through the viral cycle and, in some cases, this de-regulation of the immune system allows the persistence of the infection representing a critical event in the carcinogenic process.

Recent findings, suggest that the AT-hook transcriptional factor AKNA, could play a role in the development of cervical cancer. This factor is strongly related to the expression of co-stimulatory molecules like CD40/CD40L in order to achieve an efficient immune response. Despite all acquired data in the past years, the complete regulatory mechanism for persistent infection remains unknown and, to date, there is no evidence linking the HPV oncoproteins with the AT-hook factor AKNA. In this work, we show the ability of E6 from HPV type 18 to bind to one of the isoforms of AKNA in a series of in vitro binding assays. We also demonstrate a minimal expression of this factor and its co-stimulatory molecules in the HPV positive cell lines HeLa and SiHa in comparison with the HPV negative cell lines. The ablation of E6 in HeLa cells strongly restores p53 expression and surprisingly we detected CD40 recovery after silencing E6. This results indicate that de-regulation of CD40 and AKNA might be a common event in the carcinogenic process induced by HPV.

Characterising T cell dysfunction in human colorectal cancer

Julia Leman[1], John McCall[1], Sam Norton[1], Fran Munro[1], Helen McGuire[2], Roslyn Kemp[1]

[1]University of Otago, Dunedin, New Zealand
[2]University of Sydney, Sydney, Australia
julialeman99@gmail.com; john.mccall@otago.ac.nz; Samuel.norton@otago.ac.nz; fran.munro@otago.ac.nz; helen.mcguire@sydney.edu.au; roslyn.kemp@otago.ac.nz

Keywords: colorectal cancer, tumor, Cytometry, PD-1, exhaustion

The immune response to CRC has been associated with both disease progression and patient outcome. T cells infiltrating the tumour of people with CRC are associated with an increase in disease free survival by mediating an anti-tumour response. However, studies in both mouse models and patients have shown that activated T cells can become unresponsive and cease to proliferate or produce cytokines. These dysfunctional T cells upregulate inhibitory receptors, which can reduce T cell activation and dampen inflammatory responses upon binding to specific ligands. Tumour cells upregulate expression of these ligands and this increases the likelihood of tumour immune evasion. Immunotherapies which block the interaction between inhibitory receptors and the tumour inhibitory ligands have been effective in treating some solid cancers, but have had variable responses in treating colorectal cancer. Tumour stage, type and lymphocytic infiltrate can compromise the efficacy of these treatments and the subsequent outcomes for patients.

Flow cytometry was used to identify markers of potential T cell dysfunction expressed by tumour-infiltrating T cells. Samples of tumour and matched non-tumour bowel from patients undergoing surgery for CRC were analysed, and the expression of inhibitory and activation markers compared. These results were used to validate the incorporation of markers of dysfunction and activation into a mass cytometry panel. Using bowel tissue samples of CRC patients, we used a 40-parameter mass cytometry panel to define dysfunctional T cell populations within the tumour. Functional analyses will corroborate these findings, with the ultimate goal of identifying target markers or populations for immune therapies.

TLR stimulation in leukemic T cells and endothelial cells induces the expression of molecules needed to accomplish transendothelial migration

Ramón Castellanos Martínez, Eduardo Vadillo, Porfirio Nava, Michael Schnoor

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico

qfb.caste12@gmail.com; evadillo@hotmail.com; pnava@cinvestav.mx; mschnoor@cinvestav.mx

Keywords: Cortactin, CXCR4, TLR, LFA-1, VLA-4, Transendothelial migration, T cell leukemia

Background and Aim: T cell acute lymphoblastic leukemia (T-ALL) is an aggressive neoplasm resulting from the malignant transformation of T cell precursors, with about 25% of cases occurring in adults and 15% in children. Despite modern chemotherapies, infiltration of the central nervous system (CNS), bone marrow (BM), and testicles by leukemic T cells is a major complication of T-ALL, promoting leukemic relapse and preventing a cure of the disease. Tissue infiltration by T-ALL cells requires extravasation that might be triggered by pro-inflammatory stimuli such as activation of toll-like receptors (TLRs). Thus, we tested if local inflammation triggered by TLR stimulation can promote T cell activation and tissue infiltration.

Methodology: The human Jurkat and the murine 6645/4 T-ALL cell lines, normal T cells from peripheral blood and primary cultures of human umbilical vein endothelial cells (HUVEC) and murine lung endothelial cells (MLEC) were stimulated overnight with the TLR ligands Poly I:C low (LMW) and high molecular weight (HMW), LPS, Flagellin, Imiquimod, ssRNA40 and ODN2006 or with the chemokine CXCL12; and expression of C-X-C chemokine receptor-4 (CXCR4), TLRs, cortactin (Cttn), hematopoietic lineage cell specific protein-1 (HS1), adhesion molecule L-selectin (CD62L) and the integrins lymphocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4) were evaluated by flow cytometry. Expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells was determined by flow cytometry. CXCL12 production by HUVEC upon stimulation with TLR ligands was analyzed by confocal immunofluorescence microscopy.

Results: Jurkat cells showed a slight increase in the expression of L-selectin, LFA-1 and VLA-4 when compared with their normal counterparts from peripheral blood (PB).

Mouse leukemic T cells significantly upregulate the expression of LFA-1 and VLA-4 upon TLR-3, 4 and 5 stimulation, while ICAM-1 expression was reduced. Interestingly, CXCR4 was significantly upregulated in both Jurkat and 6645/4 when compared with normal T cells from PB. Nevertheless, mouse 6645/4 cells showed a ten-fold increase in the expression CXCR4 upon TLR-3, 4 and 5 stimulation. TLR-2, 3, 4 and 6 were barely expressed on the surface of Jurkat cells, whereas no expression on normal T cells was detected. TLR stimulation of Jurkat cells did neither activate LFA-1 nor VLA-4, but CXCL12 stimulation led to rapid activation of VLA-4 but not LFA-1. The actin-binding protein cortactin (cttn) is involved in cytoskeleton rearrangement, cell motility and migration and tissue infiltration in leukemic B cells. Therefore, we evaluated the expression of this protein in leukemic T cells. 6645/4 cells strongly overexpressed cttn compared to normal T cells from PB, while the cortactin homologue HS1 showed no difference. Moreover, after TLR-3, 4 and 5 stimulation of 6645/4 cells expression of cttn was upregulated, while HS1 expression remained the same. In HUVEC, the expression of ICAM-1 and VCAM-1 and the production of CXCL12 were significantly upregulated after 12 hours of stimulation with TLR-2, 3, 4 and 6. Also b End. 3 cells highly upregulated the expression of VCAM-1 upon TLR-3 and 4 stimulation, while ICAM-1 expression was not induced.

Conclusions: Leukemic T cell lines express the required integrins to accomplish TEM. Moreover, they highly express CXCR4 and cttn that are required for TEM and organ infiltration by controlling cytoskeleton remodeling and activation of adhesion molecules. T-ALL cells and endothelial cells also express TLRs that activate the production of CXCL12, a ligand for CXCR4 and chemoattractant for lymphocytes. Thus, T-ALL cells do have all prerequisites to extravasate and infiltrate organs. Whether our data also hold true in vivo will be tested by xenotransplantation models in the future.

Serum Galectin 9 levels in patients with cervical cancer

Verónica Vallejo-Ruiz[1], Patricia Laura Martínez-Morales[2], Jhovanna Galindo-Herrera[1], Ileana Conde-Rodríguez[1], Viridiana Robles-Sánchez[3], Juan Salvador Reyes-Salina[4], Victor Vazquez-Zamora[4], Adriana Aguilar Lemarroy[4], Luis Felipe Jave- Suárez[4], Aurelio López-Colombo[4], Francisco Javier Ceja-Utrera[4], Gerardo Santos-López[1], Julio Reyes-Leyva[1]

[1]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
[2]Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico City, Mexico
[3]Benemérita Universidad Autónoma de Puebla, Puebla, Mexico
[4]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
veronica_vallejo@yahoo.com; patricia.mtzm@gmail.com; jhovanna_gherrera@hotmail.com; iconde_rdz@yahoo.com.mx; viri_roblees@hotmail.com; salvador.reyessa@imss.gob.mx; ctulu1108@hotmail.com; adry.aguilar.lemarroy@gmail.com; lfjave@gmail.com; aurelio.lopezc@imss.gob.mx; vero.vallejoruiz@gail.com; gerardo.santos.lopez@gmail.com; jreyesleyva@yyahoo.com

Keywords: biomarker, cervical cancer, Cervical neoplasia, ELISA assay, galectin 9

Cervical cancer (CC) has decreased in developed countries thanks to cytology screening programs but it represents the second most common cancer in women from under-developed countries, where CC rates are expected to continue rising despite efforts to implement screening and treatment programs. CC develop via precursor lesions, called cervical intraepithelial neoplasia, that according to severity are graded from low grade squamous intraepithelial lesions (mild dysplasia) to high grade squamous epithelial lesions (severe dysplasia).

Different studies have shown the occurrences of false-negative in the cytology tests, thus, there is a need to identify biomarkers that could generate cost-effective assays for routine screening. Blood-based biomarkers may be an alternative non-invasive strategy to improve CC screening.

Galectins are a group of mammalian lectins with affinity for β-galactosides, these proteins share a conserved carbohydrate recognition domain (CRDs). Sixteen members of the galectin family have been identified. Galectins play an important role in cancer biology, several mechanisms are known to be influenced by galectins like adhesion, migration, apoptosis and differentiation.

Galectin-9 contains two non-homologous CRDs connected by a linker peptide. Galectin-9 can be expressed by epithelial cells as well as immune cells including T cells and neutrophils. Today experimental and clinical evidences support a correlation between galectin-9 expression and tumor transformation, and could be proposed as a biomarker in the diagnostic and prognostic of different malignancies.

Objective: To investigate the usefulness of serum galectin-9 (Gal-9) concentration in the diagnosis of cervical cancer.

We examine serum Galectin 9 concentration in a total of 91 women, 29 with normal diagnosis, 24 with low grade squamous intraepithelial lesions (LGSIL), 23 with high grade squamous intraepithelial lesions (HGSIL) and 21 with cervical cancer. Serum concentration of Gal-9 was evaluated by ELISA for galectin 9.

Results: Galectin-9 concentration was 3.78 ng/mL (3.0–4.65) in the group of women with normal citology, 4.08 ng/mL (3.1–6.32) in the patients with LGSIL, 4.1 ng/mL (3.07–5.40) in the patients with HGSIL, 5.19 ng/mL (3.59–8.41) in the patients with CC.

Conclusion: Our results suggest that Galectin-9 could be a promising noninvasive biomarker of cervical cancer. Galectin-9 could be a biomarker in CC disease monitoring, but further longitudinal evaluation is required to investigate the value of Galectin-9 serum levels in CC patients as a predictor of treatment response and prognosis.

Exposure to hypoxia induces the secretion of CCL2 chemokine in mast cells activating signaling pathways that are shared by monomeric IgE

Itzel Guadalupe Ramírez-Moreno, Alfredo Ibarra-Sánchez, Claudia González-Espinosa

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
itzel.irm@gmail.com; alfreedoibarra@gmail.com; cgonzal@cinvestav.mx

Keywords: Mast Cells, tumor, IgE, hypoxia, CCL2

Hypoxia is an important feature of malignant solid tumors and other inflammatory conditions associated with an increase in the recruitment of immune cells. Although mast cells (MCs) are important players in those inflammatory reactions, the effects of hypoxia on the cytokine profile produced by MCs has not been fully explored. In this work, we utilized in vivo and in vitro approaches to investigate, first, if MCs are located on hypoxic or normoxic zones of solid tumors and, second, the effects of hypoxia in the cytokine profile secreted by this cell type. Utilizing the murine B16 melanoma model and hypoxyprobe to localize the low-oxygen zones of the tumor, we found that an important number of tryptase-positive MCs are present in hypoxic areas of this solid tumor. On the other hand, we utilized bone marrow-derived mast cells (BMMCs) subjected to a 1% O2 atmosphere to characterize the production of specific cytokines and chemokines by this cell type in hypoxic conditions. BMMCs subjected to hypoxia were able to produce the potent chemokine CCL2. This chemokine production was sensitive to the antioxidant Trolox and the IKK inhibitor BAY117085. Interestingly, CCL2 production triggered by hypoxia was also sensitive to calcium chelator BAPTA and the L-type calcium channel blocker Nifedipine.

Since MCs have been shown to promote tumor angiogenesis in response to monomeric IgE (mIgE), we decided to analyze if a similar molecular mechanism could be involved in hypoxia or mIgE induction of CCL2 chemokine. We found that monomeric IgE leads to CCL2 production in a Trolox, BAY117085, and Nifedipine-sensitive mechanism.

Our results strongly suggest that MCs are importantly recruited to hypoxic sites of melanoma tumors and that hypoxia induces the production of the chemokine CCL2 by a mechanism that require the production of reactive oxygen species (ROS) and intracellular calcium rise through the activation of L-type calcium channels.

Also, data indicate that monomeric IgE triggers a very similar response than hypoxia to lead to the production of CCL2 in MCs. Therefore, hypoxia and stimulation with mIgE share common signaling points to induce the secretion of CCL2 in mast cells.

Is the classic signaling pathway of IL-6, associated with the coordination of structure-dependent tissue-dependent regeneration in the intestine?

Gabriela Ferragut[1], Mario Huerta[2], Maria Angelica Severi[1], Deborah Juana Keszenman[1], Juan Cedano[1]

[1]University of the Republic, Montevideo, Uruguay
[2]German Cancer Consortium, German Cancer Research Center (DKFZ), Heidelberg, Germany
gferragu@fq.edu.uy; acidshell@hotmail.com; mseveri@fq.edu.uy; dkeszen@gmail.com; jcedano@unorte.edu.uy

Keywords: Epithelial Cells, colorectal cancer, IL-6, mesenchymal cells, Tissue-remodelling, IL-6Receptor

Introduction: The physiological processes necessary to maintain the homeostasis of the tissues of living beings require the coordination of different subsystems that make up the organisms. Among these subsystems, the immune system is of great importance in many processes involved in tissue repair.

Inflammation and/or tissue remodeling, which are an important part of tissue repair processes, have been well characterized. However the complexity of the processes to be regulated is reflected in the pleiotropy of the involved cytokines, making it difficult to understand them.

The maintenance of tissue functionality requires that the immune system, which is an integral part of tissue structure, regulates with precision a regenerative response proportional to the suffered damage. When we speak of tissue renewal, regeneration or repair, we refer to the ability of the tissue to recover the functional structure of a tissue after having been damaged in one of its components or structures, to different degrees, either by simple physiological wear, after a strong stress or an aggression.

In the present work we aim to gain more insight on IL-6, not only in its contribution to tissue repair via the trans signaling pathway, but also in the possible role of the classic pathway of IL-6 signaling in the renewal and tissue regeneration. For this we will focus on the cells of the intestine, because they exemplify two types of signaling linked to IL-6 and its receptors, as well as the genes involved in the formation of tissue structure. Since intestinal epithelial cells are capable of expressing mIL-6R receptors on the surface, they can use the classical route of IL-6 to promote their cell division,

whereas mesenchymal cells depend on soluble receptors for IL-6, in the Trans route, highly linked to inflammation.

Considering that the structure of the tissue is closely linked to the function for each cell type, we have analyzed whether the interaction of the signaling pathways of the immune response related to tissue regeneration and the signaling pathways that allow the tissue have a structure in the form of crypts and villi, are coordinated.

Conclusions: From the analysis of published literature on tissue repair, crossing the structural information and analyzing the involved pathways, two basic patterns are emerging that point to a different behavior for the maintenance of intestinal tissue homeostasis, which in turn, may allow us to explain some important aspects of its physiology and pathology:

a) The mesenchymal and epithelial cells perform, respectively, different functions, either linked to their barrier function or to absorption and secretion.

b) Mesenchymal cells contribute to tissue repair processes following a temporal sequence similar to the wound healing process.

c) The epithelial cells renew the tissue in a continuous way separating the region where the function of the differentiated cells perform their function of absorption and secretion from the region in which the stem cells divide to self-renew and form new epithelial tissue that will progressively differentiate to polarized epithelial cells.

d) Both processes, although they follow different patterns, are closely coordinated among them and the signaling linked to IL-6 is central in this coordination.

e) The microenvironment and the interaction with the immune system in these regeneration processes are very important, but it is much more critical in the evolution of tissue repair linked to mesenchymal cells. Thus, it is expected that pathologies such as Crohn's or inflammatory bowel disease are more linked to alterations in the pathway of tissue repair associated with mesenchymal cells than epithelial cells.

f) Since renewal and regeneration have their own structural pattern associated with the process, even when this pattern is altered in pathological conditions, each form of growth will leave its mark on the anatomopathological structure of the tissue, and can be distinguished.

Applications: This explanatory model of tissue renewal and repair patterns can provide some light, from the physiological point of view, to understand how both sub-groups of cells recover the tissue homeostasis. In addition, it can have practical applications, opening the possibility of designing differential therapeutic approaches for each pattern. It can serve us, both to try to reverse the pathology, and to try to prevent the first steps of degeneration towards autoimmune and oncological diseases.

Role of chemokines from tumor microenvironment in the macrophages infiltration and prognosis markers in colorectal cancer

Marjorie K De La Fuente[1,2], Glauben Tamara Landskron[2], Daniela Estefani Parada Venegas[2], Karen Dubois-Camacho[2], Daniela Simian[1], Maripaz Martinez[1], Diego Romero[3], Juan Carlos Roa[3], Isidora Chahuan[2], Rocio Gutierrez[2], Francisco Lopez K[1], Karin Alvarez[1], Sebastian Lopez[2], Antonella Sanguinetti[2], Mario Abedrapo[1,2], Rodrigo Quera[1], Marcela A. Hermoso[2]

[1]Clinica Las Condes, Santiago, Chile
[2]Universidad de Chile, Santiago, Chile
[3]Pontificia Universidad Católica de Chile, Santiago, Chile
mkdelafu@gmail.com; glaulandskron@ug.uchile.cl; dparadav@gmail.com; karenduboisc@gmail.com; dsimian@clinicalascondes.cl; mmartinezj@clc.cl; biobancopuc@med.puc.cl; jcroas@gmail.com; isi_chahuan@hotmail.com; rvgutierrez@hotmail.com; flopez@clinicalascondes.cl; kalvarez@clinicalascondes.cl; sebasstianlopez@gmail.com; asanguinetti@gmail.com; mabedrapo@redclinicauchile.cl; rquera@clc.cl; marcehr@gmail.com

Keywords: Chemokines, colorectal cancer (CRC), macrophages., Tumor microenvironment (TME), tumor-associated macrophages (TAMs)

Background: Tumor microenvironment correspond to the non-cancerous cells present in the tumor and proteins produced by all of the cells present in the tumor that support the carcinogenesis, growth of the cancer cells, and metastasis. An important part of this microenviroment are immune system cells, such as macrophages, neutrophils, mast cells and T and B cells, which release inflammatory mediators such as cytokines and chemokines. According to the important role of chemokines in chemotaxis, cell trafficking and inflammation, a complex network of these molecules can influence cancer progression and tumor-associated macrophages (TAMs) infiltration. The role chemokine in TAMs infiltration and tumor progression in colorectal cancer (CCR) has not be clarified yet.

Aim: To investigate tissue and plasma levels of chemokines involved in macrophages recruitment and their association with markers of colon cancer progression.

Methodology: To evaluate the chemokine levels in CCR, samples from tumor and healthy mucosa were obtained from Chilean patients with colon cancer under surgery.

Protein extract from tissue were used to evaluate concentration of chemokines involved in macrophage recruitment (CCL2, CCL3, CCL4, CCL5 and CX3CL1), through Luminex assays. Macrophage markers (CD68, CD163, iNOS) in tumor and healthy mucosa from patients were identified through immunohistochemistry. Plasma levels of chemokines and cytokines associated to cancer (CCL2, CCL3, CCL4, TNF-a and IFN-g) were evaluated by Luminex assays in patients and controls subjects. Correlation between different markes studied were analyzed by Pearson or Spearman test, $p < 0,05$.

Results: Tumor levels of CCL2 (Median= 216. 90 pg/mg), CCL3 (Median= 67. 63 pg/mg) and CCL4 (Median= 46. 30 pg/mg) were significantly higher than those in healthy tissue (Median= 154. 40, 23. 00 and 24. 11 pg/mg, respectively). Differences in CCL5 and CX3CL1 were not observed among tumor and healthy tissue. CCL4 tumor levels shown a direct correlation with CD163 marker content in tumor from patients. The analysis of plasma levels of chemokines and cytokines in healthy controls and CRC patients show that CCL3 levels was higher in patients compared to controls (Mann Whitney test, $p < 0,05$). Moreover, CCL3 and CCL4 plasma levels were directly correlated with VEGF plasma levels in patients, that was previously associated with worse prognosis.

Conclusions: High expression of CCL2, CCL3 and CCL4 in colon cancer could induce the infiltration of TAMs. Specifically, tumor levels CCL4 correlate directly with macrophage CD163+, whereas plasma CCL4 levels correlate with a bad prognosis marker, representing a potential therapeutic target.

Expression, purification and functional characterization of SCFV antibody 3F12E7 targeting FGF2 expressed in a murine tumor model

Bruno Andrade Costa, Tábata De Almeida Da Silva, Marcelo Ferreira Marcondes Machado, Vitor Marcelo Silveira Bueno Brandão De Oliveira, Rodrigo Barbosa De Aguiar, Jane Zveiter De Moraes

Federal University of São Paulo, São Paulo, Brazil

brunofarm@hotmail.com; tabata25almeida@gmil.com; marcelo.marcondes@unifesp.com.br; vitor.oliveira@unifesp.br; rodrigo.aguiar@uol.com.br; janezvmo@gmail.com

Keywords: Monoclonal antibody (mAb), B16-F10 cells, Single chain fragment variable (scFv), Antiangiogenic immunotherapy, fibroblast growth factor (FGF) 2

Evidences suggest that fibroblast growth factor (FGF) 2 plays an important role in tumor growth and metastasis formation. Antiangiogenic immunotherapy, associated with cytotoxic therapies, has already shown its potential and has had its use approved by the FDA for the treatment of some tumors. Among the antiangiogenic drugs, there are the monoclonal antibodies. Our laboratory recently developed the anti-FGF2 monoclonal antibody (mAb) 3F12E7, which has been shown to inhibit tumor growth and metastasis formation in preclinical studies. We proposed the construction of the single chain fragment variable (scFv) 3F12E7. scFv is a protein containing the heavy (H) and light (L) variable (V) regions of the immunoglobulin bound by a linker. Due to its small size, antibodies in the form of scFv are supposed to more easily infiltrate solid tumor masses. In addition to this great advantage, compared to full-length antibody, scFv, when injected into humans, does not elicit immunological responses against the iso and allotipic markers present in the constant portion of the immunoglobulin chains. The 3F12E7 scFv was constructed from the isolation of the VH and VL genes from the 3F12E7 hybridoma. Mammalian HEK-293 cells were transformed by pCDNA3.1(+)_3F12E7 vector in order to enable the characterization of the scFv 3F12E7. The scFv 3F12E7 expression was confirmed by immunofluorescence assay performed in transformed cells treated or not with FGF2 before rabbit anti-FGF2 polyclonal antibody incubation. Escherichia coli BL21(DE3)pLysS transformed by pET_26(b)+_3F12E7 plasmid allowed further scFv 3F12E7 protein purification. The protein was expressed in inclusion bodies, that were solubilized in 8 M urea before purification by nickel-Sepharose FF resin chromatography. For refolding, the protein was dialyzed against water and, then, phosphate buffered saline (PBS), always at 4ºC. The scFv 3F12E7 protein remained stable at 37ºC for at least 48 hours and also after biotin labelling. Biotin-labeled scFv 3F12E7 recognized FGF2 on solid phase (ELISA)

and on B16-F10 tumor extracts (western blot). For the in vivo assays, B16-F10 cells were implanted subcutaneously into the left flank of the C57Bl/6 mice (5x105/animal). After 4 days, animals started being treated with scFv 3F12E7, mAb 3F12E7 or 1F5H2 isotype control mAb (8 microNormal; n=6/group) at each 48 hours until the 10th day after the implantation of the cells. Tumor growth was monitored daily with the aid of a digital caliper. On the 12th day, the animals were euthanized and the tumors removed and weighed. The following mean tumor weight ± SD (g) values were verified in each group: scFv 3F12E7: 0.334 ± 0.071; mAb 3F12E7: 0.431 ± 0.074 and 1F5H2 isotype control mAb: 0.722 ± 0.092 (ANOVA / Bonferroni post-test, $p < 0.05$ compared to control). There was no significant difference between scFv 3F12E7 and 3F12E7 full-length mAb groups. In summary, the scFv 3F12E7 has been satisfactorily expressed in mammalian cells and has maintained its ability to recognize FGF2. Labeling procedures did not impair the binding of the purified scFv 3F12E7 to FGF2. Results showed that scFv 3F12E7 activities were comparable to those observed with the mAb 3F12E7 and that scFv 3F12E7 may be potentially useful as a reagent for therapeutic purposes. It should be noted that the in vivo findings were very disappointed, since it counteracted the expectation that a smaller molecule could more easily penetrate the tumor mass and thereby bring about more expressive results than those obtained with the whole molecule of the antibody. Further studies on the solubility conditions of the purified scFv 3F12E7 may help us explain the results.

Peripheral blood leukocytes populations and inflammatory markers as a prognostic tools for breast cancer patients during neoadjuvant chemotherapy

Javier Valdes[1], Natalia Munoz[1,2], Alejandra Perez[3], Sabrina Muñoz[4], Cesar Sanchez[3], Alexis M Kalergis[1,2]

[1]Pontificia Universidad Católica de Chile, Santiago, Chile
[2]Millennium Institute on Immunology and Immunotherapy (MIII), Santiago, Chile
[3]Departamento de Endocrinología, Escuela de Medicina, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[4]Hospital de Niños Dr. Roberto del Río, Santiago, Chile
javaldes1@uc.cl; natydurango@gmail.com; aleperezs@gmail.com; msabrinamm2@gmail.com; cgsanchezr@gmail.com; akalergis@bio.puc.cl

Keywords: Anthracyclines, Chemotherapy, Adjuvant, Leukocytes, breast cancer, taxanes, Flow cytometry analysis, Monocytes Subsets, monocytes and macrophages

Worldwide, breast cancer (BC) is the main cause of cancer death among women. Chemotherapy is commonly used to control tumor growth in BC patients. For some patients chemotherapy is applied prior to resection surgery, this type of therapy is called neoadjuvant chemotherapy and aims to decrease tumor size, and increase chances of an effective tumor resection. However, in certain cases neoadjuvant chemotherapy fails in patients that have already completed their treatments, exposing the patient to an unnecessary and expensive experience. Recently, we demonstrated that the Neutrophil to Lymphocyte ratio (NLR) is a marker that determines survival in certain malignancies including BC. Our long-term goal is to develop new follow-up tools to monitor patient response to neoadjuvant chemotherapy. Here we propose to measure the abundance of different subsets of leukocytes and soluble cytokines in peripheral blood of patients as an indicator of chemotherapy efficacy. We report 20 BC patients aged 40–56 yr-old, enrolled at Hospital Sótero de Río. Patients signed written informed consents according to ethics approval. Blood samples were collected prior to neoadjuvant chemotherapy (basal state) and along 7 cycles of therapy. Initially, mononuclear cells (PBMC) were obtained to characterize subsets as: CD4+, CD8+, NKs, NKTS and Monocytes, using flow cytometry. Blood samples were also used to measure plasma pro-inflammatory markers by ELISA. Preliminary results show that lymphocytes decrease during chemotherapy. We divided our patients into two groups according to their CD4+/CD8+ T cells ratios. A first group with no changes, and another group with change along cycles with a slight tendency to increase CD8+ cells. On the other hand, the monocyte subset

showed significant differences between cycles, not only in the percentage of cells, but also in the surface expression of chemokine receptors CCR2 and CCR4. These changes occurred after patients switched their chemotherapy drugs. NKs and NKTS did not change throughout treatment. Interestingly, when taxanes were started at the 5th cycle, the NLR ratio of patients decreased, the opposite was observed with Anthracyclines. We speculate that the differences in monocytes could be used as a novel indicator on the effect of chemotherapy in patients. Finally, Taxanes are known to promote differentiation of monocytes into M1-macrophages, therefore, those cells could also be used as potential indicator of treatment effectiveness.

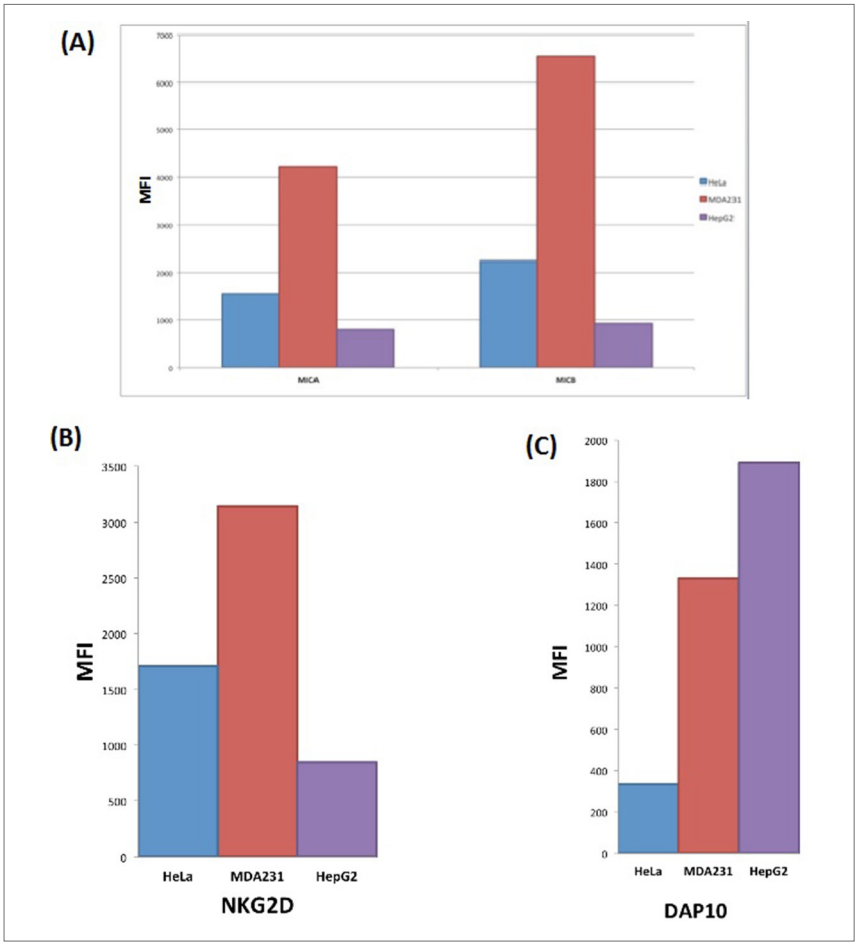
MICA regulates the expression of DAP10 and does not increase the phosphorylation of Akt in NKG2D positive cervical cancer cells

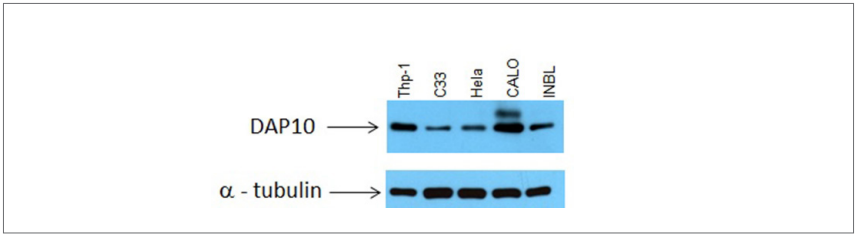
Isabel Soto-Cruz[1], Octavio Zerecero-Carreon[1], Francisco Trejo-Islas[1], Jose Luis Ventura-Gallegos[2], Alejandro Zentella-Dehesa[2], Keyra Quetzal Ramírez-Menes[1], Benny Weiss-Steider[1], Jorge Flavio Mendoza-Rincón[1]

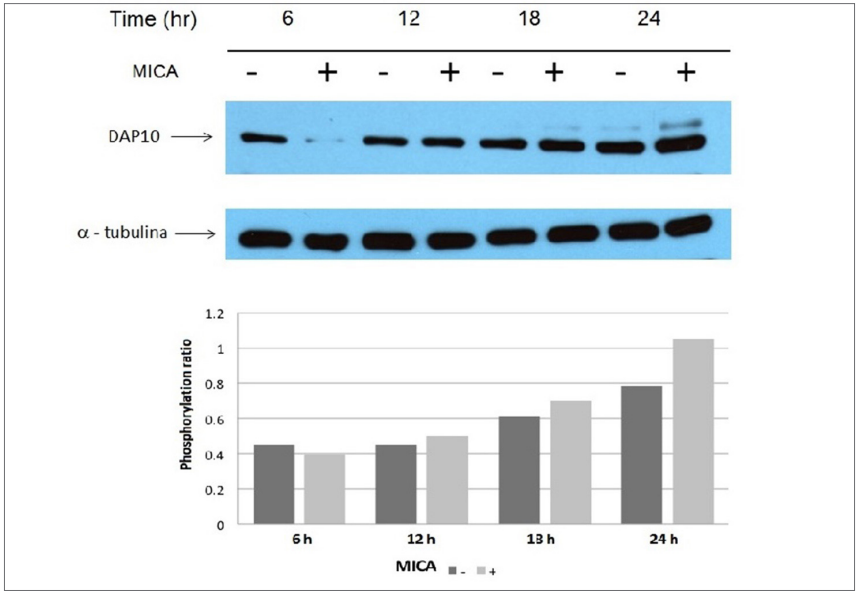
[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
sotocruz@unam.mx; octaviozerecero@gmail.com; jflavio.m@gmail.com; benturag@biomedicas.unam.mx; azentell@biomedicas.unam.mx; keytzal@gmail.com; bennyweiss@hotmail.com; jflavio@unam.mx

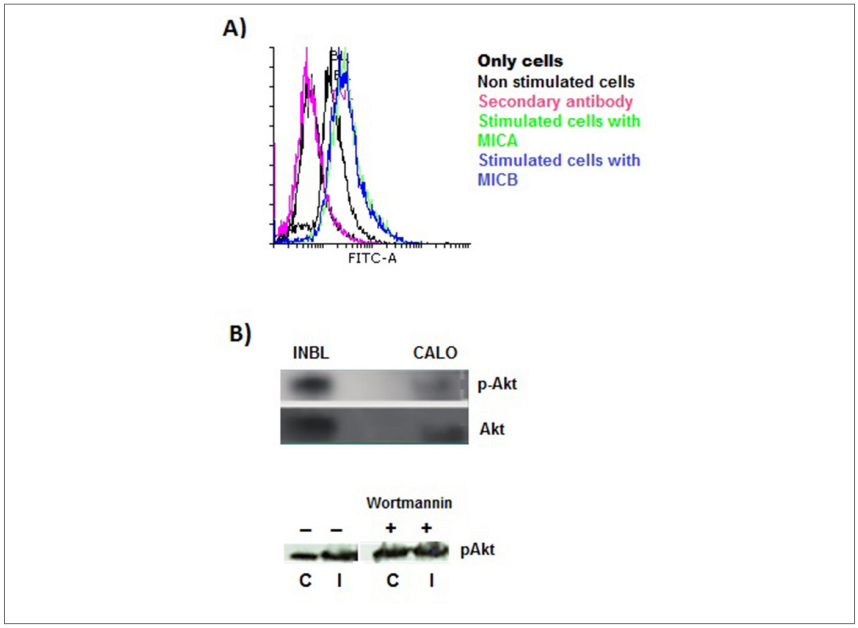
Keywords: Akt, cervical cancer, PI3K, NKG2D, MICA, DAP10

NKG2D receptor engages ligands such as MICA and MICB, which activates cytotoxicity in NK cells leading to the destruction of tumour cells expressing these ligands. In normal human lymphoid cells the association of DAP10 with NKG2D is essential for signalling and important for its cell surface expression. However, the mechanism of the NKG2D/DAP10 complex upregulation is not completely understood in cancer. Also, the role of DAP10 in the activation of the PI3/Akt signaling pathway in cervical cancer has not been fully elucidated. In the present study we investigated the role of MICA in the regulation of DAP10 in cervical cancer cells. First, we demonstrate the presence of the NKG2D/DAP10 complex in different tumour cell lines by flow cytometry. Also, we demonstrate that MICA upregulates the expression of DAP10 in cervical cancer cells in a time dependent manner by immunoblotting. We found that the Akt kinase is constitutively phosphorylated and MICA induced an increase in tyrosine phosphorylation. Furthermore, this activation is independent of the PI3K in cervical cancer cell lines as determined by immunoblotting and flow cytometry. Our results provide evidence supporting the notion that MICA functions as a stimulatory molecule to regulate the expression of the receptor adapter DAP10 in cervical cancer cells and thus may contribute to their proliferation and survival. The possibility that the NKG2D-DAP10 complex is widely expressed in different types of cancer may confer an advantage to transformed cells to survive in the tumour microenvironment and escape from the immune surveillance.


(A)
MFI
7000
6000
5000
4000
3000
2000
1000
0
MICA
MICB
HeLa
MDA231
HepG2
(B)
MFI
3500
3000
2500
2000
1500
1000
500
0
HeLa
MDA231
HepG2
NKG2D
(C)
MFI
2000
1800
1600
1400
1200
1000
800
600
400
200
0
HeLa
MDA231
HepG2
DAP10


Thp-1
C33
Hela
CALO
INBL
DAP10
α - tubulin


Time (hr)
6
12
18
24
MICA
- + - + - + - +
DAP10
α - tubulina
Phosphorylation ratio
1.2
1
0.8
0.6
0.4
0.2
0
6 h
12 h
18 h
24 h
MICA - +


A)
Only cells
Non stimulated cells
Secondary antibody
Stimulated cells with MICA
Stimulated cells with MICB
FITC-A
B)
INBL
CALO
p-Akt
Akt
Wortmannin
- - + +
pAkt
C I C I

A pro-inflammatory microenvironment promotion by tumor miRNAS in leukemic bone marrow

Jussara Rios De Los Rios[1], Jennifer Enciso[1,2], Dalia Ramirez Ramirez[1], Armando Vilchis-Ordoñez[1,3], Ricardo Vázquez-Ramírez[4], Gustavo Pedraza-Alva[5], Martha Ruiz-Tachiquín[6], Ericka Pompa-Mera[7], Alfonso Reyes-López[3], Luis Mendoza[4], Eduardo Garcia Zepeda[4], Muller Fabbri[8], Rosana Pelayo[1]

[1]Oncoimmunology Lab, CIBIOR, IMSS, Puebla, Mexico
[2]Center for Complexity Science, Mexico City, Mexico
[3]"Federico Gómez" Children's Hospital, Mexico City, Mexico
[4]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[5]Instituto de Biotecnología, UNAM, Morelos, Mexico
[6]Unidad de Investigación Médica en Genética, IMSS, Mexico City, Mexico
[7]Unidad de Investigación en Enfermedades Infecciosas y Parasitarias, Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[8]Children's Hospital of Los Angeles, Los Angeles, United States
jussara89@hotmail.com; jenny_gr8@msn.com; dal2_r@hotmail.com; vilchisarmando82@hotmail.com; ricardo1v@yahoo.com; gustavo@ibt.unam.mx; mertachiquin@yahoo.com.mx; erickanelly@yahoo.com.mx; alreypez@hotmail.com; lmendoza@biomedicas.unam.mx; garciaze@servidor.unam.mx; mfabbri@chla.usc.edu; rosanapelayo@gmail.com

Keywords: Inflammation, Leukemia, Tumor Microenvironment, miRNAs, TLR8

Introduction: Recent findings in our laboratory point towards abnormally high production of microenvironmental pro-inflammatory elements within the bone marrow (BM) from some patients at Acute Lymphoblastic Leukemia (ALL) diagnosis. The mechanism underlying this phenomenon is uncertain. The recent finding of miRNAs as hTLR8 ligands opens the possibility that microRNAs co-participate in the induction and maintenance of pro-inflammatory tumor microenvironments.

Objective: To determine the contribution of BM leukemic precursors in the establishment of pro-inflammatory microenvironments through the release of miRNAs that function as TLR8 ligands.

Material and Methods: Synthetic miRNAs that have been identified in silico as potential candidates for TLR8 ligation were co-cultured with mononuclear cells. Their capacity to bind TLR8 was assessed by immunoprecipitation of the flagged receptor. NF-kB activation was evaluated 2 h later by immunofluorescence and 12–48h hours later by

a reporter assay. Cell culture supernatants were collected at 24 h and inflammatory cytokine and growth factors levels evaluated by Milliplex assay. Supernatants were used to assess their ability of inducing proliferation of BM ALL cells. Exosome purification from ALL culture supernatants by size exclusion chromatography was performed to evaluate the secretion of the candidate miRNAs.

Results: In silico and sequence analysis suggest that at least three miRNAs are highly produced in ALL and strong inductors of pro-inflammatory factors in hematopoietic BM cells by inducing the activation of TLR signaling pathway. The three miRNAs were naturally secreted in exosomes by leukemic cells and showed their functional capabilities as TLR8 agonist ligands by activating the TLR signaling pathway, resulting in the substantial induction of pro-inflammatory factors. Co-culture of MNCs with these miRNAs resulted in NF-kB phosphorylation and the production of increased amounts of G-CSF, IL-10, IL-1b and TNF-a. Of note, co-culture of miRNA-derived supernatants with cells from bone marrow aspirates from patients with ALL induced substantial increases in the proliferation of a conspicuous CD34High primitive population.

Conclusion: We have identified miRNAs as tumor components contributing leukemic maintenance/progression by switching on pro-inflammatory pathways that further induce activation of progenitor cell populations.

Immunoregulatory role of bone marrow mesenchymal stromal cells is partially disrupted in acute leukemias

Gabriela Zamora Herrera[1,2], Adolfo García Ramírez[1,3], Blanca Félix Bañuelos García[1], Lilia Adela García Stivalet[1], Jose Alejandro Limon[1], Rosana Pelayo[1], Juan Carlos Balandrán[1,4]

[1]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
[2]*Benemérita Universidad Autónoma de Puebla, Puebla, Mexico*
[3]*Universidad Popular Autónoma del Estado de Puebla, Puebla, Mexico*
[4]*Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico*
gabriela.zamorah@alumno.buap.mx; adolfo.garcia01@upaep.edu.mx; pipucho_sol@hotmail.com; lilia.garcia@imss.gob.mx; jose.limonf@imss.gob.mx; rosanapelayo@gmail.com; jcbalandran@gmail.com

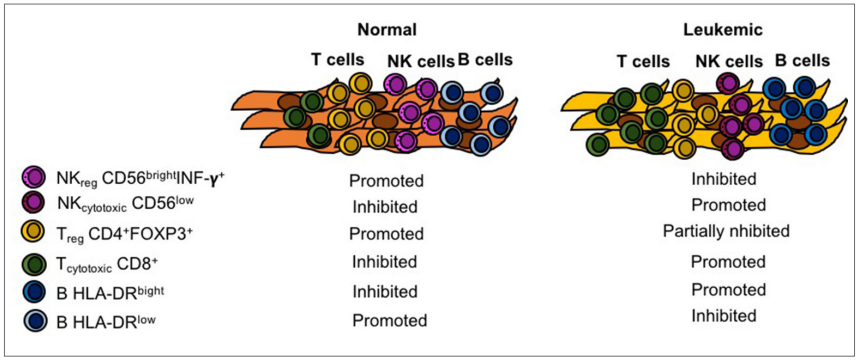
Keywords: Tumor Microenvironment, regulatory T cells, Acute leukemia, Bone marrow microenvironment, regulatory NK cells, immunoregulatory mesenchymal stromal cells

Introduction: Cancer is the major cause of death worldwide. Of all types of tumors, leukemias are the most frequent in pediatrics. Acute leukemias are a heterogeneous group of proliferative hematopoietic disease characterized by the uncontrolled cellular production of myeloid or lymphoid precursors in bone marrow (BM). High rates of malignant proliferation usually displace normal hematopoietic cells and cause the low production of normal immune cells. Our previous work has reported a pro-inflammatory microenvironment driven by leukemic cells impacting particularly the mesenchymal stromal compartment. In health conditions, mesenchymal stromal cells (MSCs) creates specialized BM niches able to support early lymphopoiesis releasing CXCL12, SCF and IL-7 and through direct cell-cell contact. MSC populations have been shown to have immunomodulatory properties and recent studies have shown that this capacity is edited during oncologic proceses. Normal MSC act in both innate and adaptive immunity by repressing T cells, suppressing dendritic cells maturation, reducing activation and proliferation of B cells, inhibiting proliferation and cytotoxicity of NK cells, and promoting regulatory T and NK cells. Their immunosuppressive properties may allow the proper hematopoietic differentiation to produce blood cells throughout life, but less is known about their role in anti-tumor surveillance or malignant progression. MSC can become immunosuppressive in response to pro-inflammatory cytokines such as IFN-γ, TNF-α, and IL-1β, and upon ligation of TLRs expressed on their surface. The net effect is due to production of immunomodulatory molecules such as IDO, PGE-2, TGF-β, IL-10 and nitric oxide, and indirectly via regeneration and expansion of potent immunosuppresor cells such as CD4+FOXP3+ and myeloid-derived regulatory

cells, including dendritic cells, monocytes/macrophages and granulocytes. MSC have been shown to induce changes in the maturation and function of normal APCs, including reduced expression of MHC class I and II antigens and costimulatory molecules, resulting in APCs unable to support T cell response. On the other hand, recent studies suggest a dual behavior depending on the stimuli of their microenvironment. As leukemia starts and progresses in the BM and MSC are critical to create leukemic niches, it will be imperative to study the biological influence of leukemic-mesenchymal niches over the immune cell populations dynamics. Recently, our group have reported a pro-inflammatory MSC scenario in B-ALL at clinical debut promoted by leukemic cells which is detrimental for normal early hematopoiesis. However, its impact on functional activities of innate and adaptive immune cells remain unraveled. A long term immunosuppressive microenvironment may benefit tumor growth even after therapy, enhancing MRD+ and reducing the possibility of total remission.

Methods: OP9 BM stromal cell were stimulated with supernatants obtained from RS4;11 (human B-ALL leukemic cell line) to stablish an experimental leukemic-mesenchymal microenvironment (ALL-OP9). On the other hand, primary human MSC were isolated from BM aspirates samples from acute leukemia patients at their clinical debut or during follow-up after treatment (MRD) by their adherent properties and were characterized by their classic immunophenotype (CD73+CD90+CD105+). Normal MSC were obtained from pediatric donors submitted to a hip surgery after informed consent of their parents. Peripheral blood mononuclear cells (PBMC) from normal donors were polyclonally activated with PMA, ionomycin and human recombinant IL-2, IL-12 and IL-18 and co-cultivated with OP-9 stroma controls or leukemic-OP9 stromal cells. PBMC were previously labeled with Carboxyfluorescein (CFSE) to assay proliferation by flow cytometry. After one week of co-culture, cells were harvested and immunophenotype for TCD4+, TCD8+, Treg, B, NK and NKT were investigated. Same experiments were conducted using primary human MSC. Differences in proliferation rates, phenotypes and cellular frequencies are reported.

Result: OP9 cells were able to attenuate lymphocytes activation at the time to decrease expression of MHC II Class on B cells and inhibit INF-gamm production by NK cells. Interestingly, ALL-OP9 cannot be able to turn off immune activation as strong as in controls. In addition, we note the emergency of T CD8low cells and the vast expansion of NK cells CD56dim suggesting a cytotoxic role. Recent data from our lab have found that regulatory NK cells (CD56brightIL-10+) can be promoted by MSC isolated from B-ALL patients with very poor prognosis (relapsed). It has been shown that TGF-beta, IL-10, IL-15 are critical to promote NKreg phenotype. Experiments by adding human recombinant IL-15 will be critical because OP9 cannot produce it. In consequence,



cytotoxic assays or IL-10/INF-gamma staining should be also conducted to evaluate both functions. It is known that normal MSC inhibit activation of T cells but enhancing differentiation of Treg population CD3+CD4+Foxp3+ (Fig.1). It is uncertain so far whether the generation of this regulatory populations are natural or induced by the microenvironment or other immune populations present in co-cultures. Emerging of Treg in ALL-OP9 were partially reduced (~25%) compared to controls. Interestingly, ALL-OP9 allowed maintenance of T DN cells (CD3+CD44+CD4-CD8-), this may be sustained by the overexpression of Jagged-1 and DLL-4 in MSC during some leukemia context. If we can mimic normal and leukemic niches in vitro we will be able to study interaction between them and explore the tumor edition phenomenon. Investigation of the molecular mechanisms of MSC to promote/inhibit suppressor immune cells may contribute new prognostic parameters for hematological patients.

Preliminar Conclusion: At clinical debut, BM microenvironment is remodeled by leukemic cells creating a pro-inflammatory environment unable to support normal lymphopoiesis. Remaining normal niches should be responsible to continue the production of immune cells. However, the long term immunosuppressive phenotype could contribute to cancer edition. Our work suggests that their immunoregulatory properties are modified in leukemia allowing activation of innate responses, specially from NK cells. Strikingly, Tregs in BM were predominantly high in BM of relapse leukemias, while low frequencies were found after culture in those patients with complete remission and in healthy donors. Unraveling the mechanisms behind the support of immunosupressive cells in the various phases of leukemia may have clinical value.

Transcriptional expression of APC, K-Ras and β-Cathenin and in a chemically induced murine model of colon cancer

María Del Rosario Huizar-López, Karen Alejandra González-Aguilar, Patricia Castro-Félix, Josefina Casas-Solís, Anne Santerre

Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
mhuizarl@yahoo.com.mx; kagen1ce@hotmail.com; patriciacastrofelix@gmail.com; jcasasmicro@gmail.com; asanterre@live.com.mx

Keywords: Colon, Cancer, APC, mouse models, K-ras, AOM-DSS

Introduction: According to the Global Health Observatory, 13% of global mortality is due to cancer. Among those, colorectal cancer (CC), a malignant tumor of the gastrointestinal tract, represents the fourth most common cause of cancer-related death worldwide (Kim et al., 2015). Colorectal cancer is a multi-step process and multifactorial disease, with genetic, environmental, dietary and life-style risk factors. In Mexico, CC is the fourth type of cancer and its incidence is growing because of the adoption of sedentary lifestyle and Western diets. Colorectal cancer is mostly detected in mid/late adulthood, beyond the fifth decade of life; however, cases of early onsets of CC have recently increased. An early detection of CC is associated with up to 90% rate of survival, therefore, it is important to implement early CC screening and treatment schemes, and above all to design and apply prevention protocols to reduce its incidence. The first events of CC take place in the colon epithelium, which normally forms an impermeable barrier covered with a mucus layer that defends the intestinal wall from damaging pathogens, harmful chemicals, and physical injuries. At molecular level, colon carcinogenesis involves changes associated with the accumulation of somatic mutations as well as epigenetic alterations. These modifications confer cells within the colonic crypts, advantages for division and transformation (Rodríguez, 2012). Despite strong hereditary and familiar components, most CC cases (>95%) are sporadic and develop slowly over several years. Fearon and Vogelstein (1990) proposed that the progression of CC is the result of the accumulation of mutations in more than one gene (Juárez-Vázquez and Rosales-Reynoso, 2014). In particular the mutational activation of the K-Ras (Kristen rat sarcoma viral oncogene homolog) oncogene and high nuclear levels of the oncogenic protein β-cathenin, together with the inactivation of Apc (Adenomatous polyposis coli) and deletions in DNA repair genes have been involved in colon carcinogenesis (Mantilla et al., 2015).

Chemically induced murine experimental models provide an excellent tool for the study of the molecular mechanisms of CC (Tong et al., 2011) and offer the opportunity to test the protective effect of plant extracts (Huizar-Lopez et al., 2018) and probiotics against CC (Irecta-Nájera et al., 2017). As dietary supplements, these compounds may prevent CC and act in a less invasive, more efficient and more specific way, and with less negative side effects than classical treatment schemes.

The aim of the study was to evaluate the transcriptional expression of K-Ras, Apc and β-cathenin in a murine colon cancer model induced with azoxymethane and dextran sulfate sodium salt by using RT-qPCR.

Materials and Methods: Female BALB/c mice 14–16 weeks old (25 ± 2 g in weight) were used. The combination of azoxymethane (AOM) with dextran sulfate sodium salt (DSS) was used as a model for colitis associated cancer. Briefly the protocol consisted in the administration of the genotoxic agent AOM (Sigma-Aldrich,10 mg/Kg of body weight, two i.p. injections) followed by two five-day cycles of the pro-inflammatory agent DSS (2% in drinking water) (MP Biomedicals) (Tanaka et al., 2003; Thaker et al., 2012). The experiment design included the healthy control, AOM alone and AOM+DSS groups (n=6). Mice were sacrificed at week 15 and distal colon was obtained. Total RNA was extracted using Trizol reagent. Complementary DNA (cDNA) was synthesized using oligo dT12-18 and M-MVL retrotranscriptase (Invitrogen). Quantity and integrity of the RNA were checked with Nanodrop. Specific EPIC primers (Li et al., 2010) for Apc, K-Ras, β-cathenin and GAPDH were designed; and synthesized by Integrated DNA Technologies (IDT). Quantitative real-time PCR (RT-qPCR) reactions were carried out using SYBR Green (Thermo Scientific). The experiments were performed in a StepOnePlus instrument (BD). Relative gene expression was calculated according to the ΔΔCt method (Schmittgen and Livak, 2008). One way ANOVA and Post hoc SNK or the Kruskal Wallis tests were applied; $p < 0.05$ indicates significant differences.

Result: Figure 1 shows that the relative expression of Apc decreased significantly in the AOM+DSS group compared to the healthy control. The expression of Apc also markedly diminished in the AOM group compared to control. Figure 2 shows that the relative expression of K-Ras tended to increase in the AOM and AOM+DSS groups compared to the control. Figure 3 shows that the expression of β-cathenin did not change.

Discussion: The experimental data indicate that the transcriptional expression of the tumor suppressor gene Apc was affected by the treatment with AOM alone or in combination with DSS. This data coincides with a report from Fajardo and Piazza (2015) in

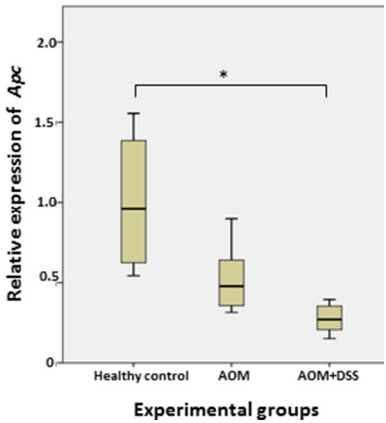


FIGURE 1: Relative expression of *Apc* in distal colon of Balb/c mice. 1) Healthy control, 2) AOM alone, 3) AOM + DSS. One way ANOVA, * indicates significance differences ($p<0.05$) between the AOM+DSS group and the Healthy Control group. Even though the expression of *Apc* is lower in the AOM alone group compared to the Healthy Control group, this dlfference did not reach significance.

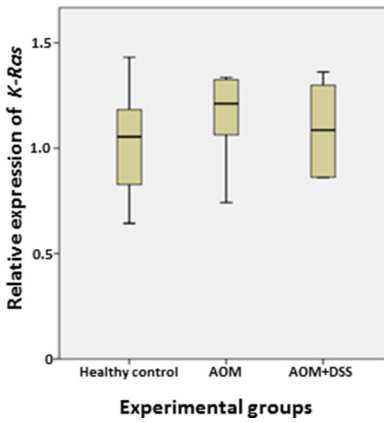


FIGURE 2: Relative expression of K-Ras in distal colon of Balb/c mice. 1) Healthy Control 2) AOM alone 3) AOM+DSS. One way ANOVA, no statistical differences were observed between study groups ($p>0.05$).

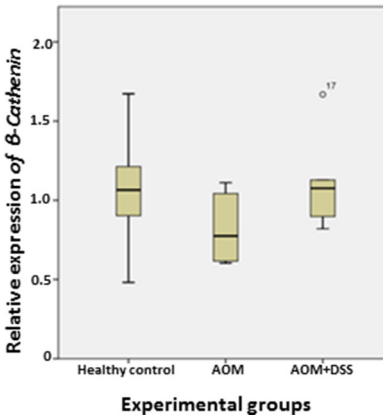


FIGURE 3: Relative expression of β-cathenin in distal colon of Balb/c mice. 1) Healthy Control, 2) AOM alone, 3) ADM + DSS. One way ANOVA, no statistical differences were observed between study groups ($p>0.05$).

CC patients who proposed that an early event of CC may be biallelic mutations of Apc and the inactivation of the corresponding protein. It was also observed that inflammation is important for CC because the administration of DSS potentiated the effect of AOM on Apc expression. The evaluation of seric levels of pro and anti-inflammatory cytokines has been undertaken.

At week 15 of experimentation, the expression of K-Ras only started to decrease in the treated groups. This may suggest that in this model the effect of the treatments on K-Ras expression is a late event in colon carcinogenesis. Furthermore, we can´t rule out that within the segment of colonic tissue used in this experiment, normal and mutated cells may still coexist, and thus the levels of expression observed here may represent an average of K-Ras expression. In further experiments we will also focus on the polyps observed in the colon of these animals.

As expected, the transcriptional expression of β-cathenin was not altered in treated groups, thus its evaluation will be further assessed by immunohistochemistry to follow the effect of the treatments on the cytoplasmic/nuclear localization of this protein for its key role in the Wnt/Apc/β-cathenin pathway (Ramos-Solano et al., 2015).

As a conclusion, the murine model presented here highlights the key role of Apc expression as an early event of colon carcinogenesis. In ongoing experiments we are now paying special attention to Apc expression to assess in this model the preventive and /or antitumoral effect of products of natural origin such as probiotics and medicinal plant extracts in colon carcinogenesis.

REFERENCES

Fajardo A.M., Piazza G.A. (2015). Chemoprevention in gastrointestinal physiology and disease. Anti-inflammatory approaches for colorectal cancer chemoprevention. American Journal of Physiology. Gastrointestinal and Liver Physiology 309(2):G59-70.

Fearon E.R., Vogelstein B. (1990). A genetic model for colorectal tumorigenesis. Cell 61(5):759-767.

Irecta-Nájera C.A., Huizar-López M., Casas-Solís J., Castro-Félix P., Santerre A. (2017). Protective effect of Lactobacillus casei on DMH-induced colon carcinogenesis in mice. Probiotics Antimicrob Proteins 9(2):163-171.

Huizar-López M.R., Alcazar-Rios I.C., Castro-Félix P., Casas-Solís J., Santerre A (2018). Cytotoxic effect of the ethanolic extracts of dry and fresh leaves of Annona muricata L. in breast cancer cell lines. Conference Abstract: XII Congress of the Latin American Association of Immunology & XXIII Congress of the Mexican Society of Immunology.

Juárez-Vázquez C.I., Rosales-Reynoso M.A. (2014). Cáncer colorrectal (CCR): alteraciones genéticas y moleculares. Universidad de Guadalajara. División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social (IMSS). Gaceta Médica de México 150:154-164.

Kim S.E., Paik H.Y., Yoon H., Lee J.E., Kim N., Sung M.K. (2015). Sex- and gender-specific disparities in colorectal cancer risk. World Journal of Gastroenterology 21(17):5167-75.

Li C., Riethoven J.J., Lingbo M. (2010). Exon-primed intron-crossing (EPIC) markers for non-model teleost fishes. Biomedical Central Evolutionary Biology 10(90):1-12.

Mantilla C., Suárez M.I., Duque-Jaramillo A., Navas M.C. (2015). Mecanismos de señalización por β-catenina y su papel en la carcinogénesis. CES Medicina 29(1):109-128.

Ramos-Solano M., Álvarez-Zavala M., García-Castro B., Jave-Suárez L.F., Aguilar-Lemarroy A. (2015). Vía de señalización Wnt y cáncer cervicouterino. Revista Médica del Instituto Mexicano del Seguro Social 53(2):S218-S224.

Rodríguez G.M. (2012). Potencial terapéutico de medicamentos antineoplásicos alternativos sobre la línea COLO 320 y cultivos primarios de adenocarcinoma colorrectal. Universidad Autónoma de Nuevo León. Facultad de Medicina 1-10.

Schmittgen T.D., Livak K.J. (2008). Analyzing real-time PCR data by the comparative CT method. Nature Protocols 3(6):1101-1109.

Tanaka T., Kohno H., Suzuki R., Yamada Y., Sugie S., Mori H. (2003). A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. Cancer Science 94(11):965-973.

Thaker A.I., Shaker A., Rao M.S., Ciorba M.A. (2012). Modeling Colitis-Associated Cancer with Azoxymethane (AOM) and Dextran Sulfate Sodium (DSS). Journal Visualized Experiment 67:e4100.

Tong Y., Yang W., Koeffler P. (2011). Mouse model of colorectal cancer. Chinese Journal of cancer 30(7):450-462.

Exosomes produced by breast cancer cells induce and expand T regulatory T cells and increase their suppressor capacity

Estefanía Scarlett Ortega Chávez, Arimelek Cortés Hernández, Cynthia López Pacheco, Soldevila Gloria, Eduardo Garcia Zepeda

Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
scarlett_ortega@outlook.es; arimelekch@hotmail.com; cyntlop@hotmail.com; soldevi@unam.mx; garciaze@servidor.unam.mx

Keywords: Exosomes, breast cancer, induction, suppression mechanisms, Treg cells, expansion

Introduction: Breast cancer (BC) is the most frequent female neoplasm in the world. Within the tumor microenvironment, there are complex interactions between tumor cells, non-tumoral cells (immune cells, endothelial cells, fibroblasts, adipocytes), and soluble factors (cytokines, chemokines, growth factors and exosomes) that favor tumor progression. An important feature of BC is the presence of a massive lymphoid infiltrate in the primary tumor, which is likely favored by inflammatory cytokines produced by tumor cells. Several studies support the idea of a local immunosuppressive environment inhibiting proper effector cell function even after effective homing. The presence of abnormal quantities of regulatory T cells (Treg) within the tumor site and the peripheral blood is a clear indication of this immune suppression. Recently, has been described a new modulator mechanism induced by exosomes (microvesicles of 30–200 nm in diameter, which contain proteins -factors of transcription, growth- and genetic material -RNA, miRNAs, DNA-) released by tumor cells, that could have a fundamental role in cancer progression. Exosomes of some tumors as of breast, prostate and glioma, have the ability to induce apoptosis (route Fas-FasL) and to diminish the cytotoxic capacity of lymphocytes. Nasopharyngeal cancer exosomes were demonstrated to induce and expand, as well as to increase their suppressive function. However, the specific mechanisms of generation and expansion of Treg cells with tumor exosomes have not yet been described.

Objective: To characterize the functional effect of exosomes produced by breast cancer cells in the conversion and expansion of regulatory T cells.

Methods: As a study model three-dimensional (3D) cell culture was used (spheroids, enriched in cells with stem-like phenotype) of the BC cell lines MCF-7 (non-metastatic), and MDA-MB-468 (metastatic). Exosomes were purified by size exclusion,

characterized by transmission electron microscopy (TEM) and quantified by NTA (Nanoparticle Tracking Analysis). For the in vitro of generation and expansion of Treg, FACS-purified naive T cells (CD4+, CD25-, CD45RA+) and Treg (CD4+, CD25HI, CD127-) of healthy individuals, were co-cultured with tumor-derived exosomes at different concentrations (50, 500, 5 000, 10 000 and 20 000 exosomes by T-cell) and expression of classic markers of regulatory T cells were evaluated by flow cytometry. Treg cells used in suppression assays were pre-incubated whit exosomes for 5 days and then co-cultured whit autologous CD4/CD8 T cells at the 1:2, 1:8 and 1:32 ratios.

Results: Phenotypic analysis of cancer cells grown in 3D showed increase in the percentage of CD24-/CD44+ in the MCF-7 line but not in the MDA-MB-468 cell line, indicating an enrichment of cells with stem-like phenotype in 3D cultures from this non metastatic BC cell line. Analysis of the exosomes from 3D cultures from MCF-7 and MDA-MB-468 by NTA and TEM showed that they have the characteristic morphology of exosomes with a size ranging from 30–200 nm and concentration of 7.6X109 and 3.9X1010 exosomes/ml respectively, obtained approximately from 3X106 cells. Analysis of in vitro induction of Treg from purified CD4+CD25- CD45RA+ naïve T cells, showed that after 4 days of culture the Treg frequency increased in the presence of exosomes obtained from MCF-7 compared with the same cells cultured in the absence of exosomes, leading to overexpression of markers associated with the Treg phenotype, such as CD25, CTLA4 and Foxp3. In addition, this effect appeared to be dose-dependent.

Furthermore, the expansion de Treg defined as CD4+ CD25HI Foxp3+ was evaluated in the 7 days, showing that exosomes from MCF-7 promoted the expansion of Treg cells, and also induced overexpression of markers CD25, CTLA4 and Foxp3, in a dose dependent manner. On the other hand, Treg expansion in the presence of exosomes derived from the MDA-MB-468 3D cultures was also able to induce enhanced Treg expansion and upregulation of CD25, CTLA-4 and Foxp3, although they appeared to reduce Treg proliferation in vitro. Interestingly, Treg cell expanded with exosomes showed higher suppressor capacity of CD4+ and CD8+ T cells, compared with the same cells co-cultured with Tregs expanded in the absence of exosomes.

Conclusion: Interactions between tumor exosomes and Tregs may be involved in the tolerance to the tumor and may support immune evasion in breast cancer. Our results demonstrate that exosomes from BC tumor cells have the ability to expand and induce Treg cells and increase their suppressor capacity, and through these mechanisms may play a fundamental role in promoting cancer progression.

In vitro evaluation of the effect of hydralazine and magnesium valproate into the chemotactic and proliferative relation of mast cell – tumor cell

Marisol Ramírez Yautentzi[1], Guadalupe Domínguez Gómez[2], Rommel Chacón-Salinas[3], Alfonso Dueñas-González[1,2], Alma Delia Chávez Blanco[2]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Nacional de Cancerología (INCan), Mexico, Mexico
[3]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico
maray_311@hotmail.com; dguadalupeisabel@yahoo.com.mx; rommelchacons@yahoo.com.mx; alfonso_duenasg@yahoo.com; celular_alma@hotmail.com

Keywords: Hydralazine, Mast Cells, Cancer, HDAC, DNMT, valproic acid (VPA)

Mast cells have a relevant role within the tumoral microenviroment. Once activated, mast cells have the ability to secrete a series of inflammatory mediators which can act in different ways a)initiate an immune response, b) attract inflammatory cells, c) mediate tissue remodeling and repair. Also, mast cells can change their phenotype depending on the duration of stimulus exposure and they can be presented in different subtypes according to the distribution of tissues, resulting in the "plasticity of mast cells". Nevertheless, the increased accumulation of mast cells has been correlated with poor prognosis, increased metastasis and reduced survival in several types of cancer, including melanoma, prostate, pancreas, lung, colon and cervical. The recruitment of these cells depends on the secretion of soluble factors derived from tumor cells, of which, the stem cell factor (SCF) is considered to be more important. In addition, chemotactic factors act on the receptors expressed by mast cells: CCL5 (RANTES), vascular endotelial growth factor (VEGF), angiopoietin 1 (Ang 1), monocyte chemoattractant protein-1 (MCP-1), CXCL8/IL-8. The increased heterogeneity of the different types of immune cells, their plasticity and their reciprocal interactions have complicated the understanding of the microenviroment at the beginning and development of the tumor. There are studies that have attempted to identify the contributions of mast cells in tumor growth; in some of them, mast cells have a pro-tumorigenic or anti-tumorigenic role. This contradictory role suggests that mas cells and their mediators in tumors may be specific to the type of cancer, however, there are few studies that evaluate other types of molecules secreted by mast cells and tumor cells, and could be of great importance. During the immunoedition of the tumor, epigenetic changes occur and stimulate the pro-tumoral activity, which leads to the search for new therapeutic targets that reverse these changes and favor the elimination of tumor cells. It has been

reported that inhibitors of DNA methyltransferases (DNMT) an histone deacetylases (HDAC) induce differentiation, apoptosis and inhibition of growth in tumor cells. Moreover, studies have been reported the effect of iHDAC and iDNMT on immune cells, favoring the immune response. In our research group, hydralazine and valproic acid has been studied as a chromatin remodelers. It has reported their synergistic effect on gene expression in different tumor line and the regulation of genes involved in the arrest of cell cycle, angiogenesis, apoptosis and immune modulation.

However, the role of these agents has not been studied within the interaction between mast cells and tumor cells. Although there are strong data tha associate mast cells as an important component in tumor progression, no molecules have been reported that may have any beneficial effect in such interaction, and therefore, can be used in cancer therapy. In this way, the aim of this Project is evaluate the biological effect of the hydralizine and valproic acid into the mast cell–tumor cell interaction. In particular, the chemotactic and proliferative capacity in the interaction between mast cell and tumor cell without treatment and treated with hydralazine and magnesium valproate will be evaluated.

Exosomes produced by metastatic breast cancer cells induce more angiogenesis than non-metastatic breast cancer cells in spheroid culture conditions

Alberto Chinney-Herrera[1], Estefanía Ortega[1], Jacobo Martínez-Ríos[1], Gisela DuPont[1], Eduardo A García-Zepeda[2], Gloria Soldevila-Melgarejo[2]

[1]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

[2]Instituto de Investigaciones Biomedicas UNAM, Ciudad de México, Mexico

alder_30@hotmail.com; scarlett_ortega@outlook.com; belerofontej@hotmail.com; gisela@biomedicas.unam.mx; garciaze@servidor.unam.mx; soldevi@servidor.unam.mx

Keywords: Exosomes, breast cancer, metastasis, Angiogenesis, Tumor spheroids

Introduction: Breast cancer (BC) is the most frequent female neoplasm in the world. Tumor cells produce a wide range of cytokines and growth factors for angiogenesis induction, which promotes disease progression. Interestingly, a new angiogenic modulator mechanism induced by exosomes (microvesicles) released by tumor cells has recently been documented; these microvesicles contain proteins and genetic material with different regulatory capacities in target cells. However, the cellular phenotype can affect exosomes composition. In different culture conditions such as adherent (2D) or spheroid (3D) a single cell line can express multiple phenotypes. Growing cells in spheroids has been reported to better resemble the phenotype of tumor cells in vivo, however it is unknown whether different subtypes of breast cancer cells and culture conditions may affect the characteristics and functions of the exosomes produced by these cells. The aim of this work is to characterize exosomes from breast cancer cell cultures and assess the angiogenic effect of microvesicles from 2D versus 3D cultures.

Methods: Exosomes were obtained by ultracentrifugation of two BC cell lines cultures supernatants: MCF-7 (non-metastatic) and MDA-MB-468 (metastatic) in 2D and 3D cultures. The exosomes were characterized by electron microscopy, and quantified by nanoparticle tracking analysis. HUVEC cells were co-cultured with an equal concentration of exosomes from different culture conditions for 6 hours. The angiogenic effect was analysed using the ImageJ software, by quantifying the number of tubules, branch sites/nodes and loops/meshes induced. bFGF was used a positive control for angiogenesis.

Results and Conclusions: The exosomes of both tumor cell lines have pro-angiogenic effect, being exosomes from 3D cultures more potent than those from 2D cultures; Nevertheless, the exosomes from MDA-MB-468 cultured in 3D conditions induced a higher pro-angiogenic effect than exosomes form MCF7 3D cultures. The fact that the same number of exosomes from 3D showed increased angiogenic properties compared to their 2D counterparts, suggest that their content may be modified. In this context, it has been shown that the generation of mamospheres correlates with an enrichment in cancer stem cells, and therefore phenotypic changes in cell composition. If this assumption is correct, diversity of microvesicle content for intercellular communication will not depend solely on cell type, but also on the influence of the tumor microenvironment, which may change during cancer progression.

Epigenetic drug 5-azacytidine enhances potential killing of CD8 T cells

Felipe Campos-de-Almeida[1], Helen Loo Yau[2,3], Isabella Suzuki Sampaio[1], Henry Alonso Paico Montero[1], Maryanne Melanie Gonzales Carazas[1], Daniel Diniz De Carvalho[2,3], Gustavo P. Amarante-Mendes[1]

[1]*University of São Paulo, São Paulo, Brazil*
[2]*Department of Medical Biophysics, University of Toronto, Toronto, Canada*
[3]*Princess Margaret Cancer Centre, University Health Network, Toronto, Canada*
fcampos13@gmail.com; hehe.looyau@mail.utoronto.ca; isuzukisampaio@gmail.com; henry_paico@hotmail.com; marymel.g@gmail.com; ddecarv@uhnresearch.ca; gpam@usp.br

Keywords: DNA Methylation, cancer therapies, CD8 T cells, Cancer cells, epigenetic therapy, anti-tumorigenic effects

Introduction: Low doses of the DNA methylation inhibitor (DNMTi), on cancer cells has durable anti-tumorigenic effects by inducing cell cycle arrest and immune-modulatory properties that increase their visibility by the immune system. Growing evidence led by our group and others suggest that DNMTis can upregulate immune signaling in cancer cells through viral mimicry and upregulation of tumour associated antigens, highlighting the clinical potential to combine with immunotherapy. While the effects of DNMTis have been mostly studied in cancer cells, their effects on the immune system remain vastly unknown, especially at clinically relevant low doses. Therefore, we aimed to investigate the effect of administration of low doses of DNA demethylating inhibitor on the effector function of CD8+ T cells.

Methods and Results: We immunized mice with recombinant adenoviral vectors, which raises a strong CD8 T cell response, along with pentamers staining and ELISPOT assay (to measure the frequency of antigen-specific CD8 T cell), the in vivo cytotoxic assay (to evaluate the in vivo elimination of antigen-specific CD8 T cell targets) and CD8 T cells immunophenotyping. Mice were treated with 5-Azacytidine (0,2 mg/kg) and PBS intraperitoneally during 7 days. We also used OT1 transgenic mice model in which the CD8+ T cells have a TCR specific for OVA peptide SIINFEKL to perform a killing assay. We expanded and treated isolated OT1 CD8+ T cells ex vivo with DAC and performed an ex vivo killing experiment against OVA-pulsed cells.
Results: Using the pentamers staining and ELISPOT assay, we found a significant decrease at the frequency of antigen-specific CD8 T cells in the DNMTi treated group

compared with the untreated animals after 14 days of immunization ($P < 0.01$). On the other hand, we did not find a significant difference between the experimental groups regarding the in vivo killing of targets by antigen-specific CD8 T cells at the same time point. Furthermore, in our in vitro killing assay, we were able to show the enhanced killing potential of the antigen-specific T cells treated with DNMTi.

Conclusion: Remarkably, while DNMTi treatment reduced T-cell expansion, the in-vivo killing capacity of these antigen-specific T cells was found to be significantly enhanced. Altogether, our results show that treatment of CD8+ T cells enhances cytolytic potential. Therefore, by studying the effects of 5-AZA-CdR on CD8+ T cells, our goal is to use this knowledge generated in here to help elucidate rational combinatorial regimens for epigenetic therapy with others cancer therapies.

Characterization of breast cancer patient-derived adiposomes and their effect on Treg generation and expansion

Cynthia Paola López Pacheco[1,2], Naray Alejandra Acevedo Domínguez[1,2], Diana Paulina Herrera Villegas[1,2], Estefanía Scarlett Ortega Chávez[1,2], Aleida Núñez Ruiz[1], Flor Trinidad Sánchez Brena[1], Alicia Rodríguez Martínez[2,3], Gregorio Quintero Beuló[3], Azcary Vázquez Tinajero[3], Fernando Lara González[3], Roberto Mosiñoz Montes[3], Eduardo Alberto García Zepeda[1,2], Soldevila Gloria[1]

[1]*Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico*
[2]*Chemokine Biology Research Laboratory, Universidad Nacional Autónoma de México, Mexico City, Mexico*
[3]*Hospital General de México, Mexico City, Mexico*
cyntlop@hotmail.com; nara_2305@hotmail.com; paulina.hevi@gmail.com; scarlett_ortega@outlook.es; aleidanuru@hotmail.com; flowerft@gmail.com; lactancia_med2013@hotmail.com; gregorioqb@prodigy.net.mx; azcaryvazquez@hotmail.com; drfernandolaraglez@gmail.com; mocinosmd@yahoo.es; garciaze@unam.mx; soldevi@unam.mx

Keywords: Obesity, breast cancer, Treg cells, exosomes and immune modulation, adiposomes

Introduction: Breast cancer (BC) is one of the most important causes of women's death worldwide. Even if many genetic and environmental unavoidable risk factors are associated with BC development and prognosis, other avoidable factors are also related to BC, as the lack of physical activity, poor diet, combined hormone replacement therapy and being overweight or obese.

Obesity is an abnormal condition that promotes fat accumulation and it has negative effects to human health. Recently, obese adipose tissue has been described as an important source of pro-inflammatory mediators and an important regulator of metabolic changes that are associated with BC. The differences between lean and obese fat concerning the infiltrating immune cells and their function have been well described. In cancer, tumor infiltrating regulatory T (Treg) cells may have a pro-tumoral role because of their suppressive function over other anti-tumor immune populations. A high number of intratumoral Treg cells have been generally correlated with poor prognosis and short overall survival in BC patients although their role remains ambiguous. This population can be recruited from the periphery, but it can also be converted from non Treg cells and expanded in situ by the tumor microenvironment. There are some known factors that can induce the expansion of these cells, including recognition of specific self-antigens by memory Treg cells. It has been demonstrated that an intimate

communication between adipocytes and tumor microenvironment is determinant to tumor progression. This association is mediated by adipocyte-secreted factors that could be transported by a novel mechanism that has been recently described and explored: adipose tissue-derived exosomes (adiposomes).

Exosomes are small microvesicles of 50–200 nm with an important content of proteins and micro-RNAs. The molecules carried by exosomes are important modulators of cancer development. In this context, it has been demonstrated that the interaction of tumor exosomes with lymphocytes in vitro, enhances Treg survival and/or proliferation, however the impact of adiposomes on Treg has been unexplored and the molecules that may be responsible for directly or indirectly promoting Treg conversion and/or expansion have not been identified.

Aim: The goal of this work is to purify and characterize adiposomes derived from tumor cancer patients and to analyze their effect on Treg induction and expansion.

Hypothesis: Tumor-associated adipose tissue from obese breast cancer patients produce adiposomes that promote Treg induction and/or expansion.

Methods: Tumor-associated adipose tissue and peripheral blood are collected from from BC-diagnosed female patients undergoing surgery for mammary gland resection. The tissue was digested with collagenase and adipocytes were collected from the oily phase, washed twice and then cultured in alpha-MEM and F-12 medium without bovine serum to avoid serum-derived exosomas interference. Supernatant was collected and clarified to reduce debris contamination. Then, supernatant was ultra-filtered and separated by molecular exclusion with sepharose. Finally, adiposomes were quantified and their size was determined by nanotracking particle analysis (NTA). Morphology was analyzed by Transmission Electron Microscopy (TEM) and protein content of tetraspanins Tsg-101, CD9, CD81 was analyzed by western blot.

To investigate the effect of these adiposomes in regulatory T-cell conversion or proliferation (expansion), peripheral blood mononuclear cells (PBMC) from patients with BC were purified from 20–50 mL blood samples and sorted by the co-expression of CD4+ CD25- CD45RAhi (naïve T cells) and CD4+ CD25hi, CD127- (regulatory T cells). Regarding the ex vivo conversion of CD4+ CD25- CD45RA+ naïve T cells to Treg cells, 20,000 cells were cultured in the presence of IL-2 (50 U/mL), Human T activator CD3/CD28 Dynabeads® in a 1 bead:10 cells ratio, and TGFβ 1 ng/mL in 200 µL of GIBCO OpTmizer™ CTS™ T-Cell Expansion SFM.

For the ex vivo expansion of the regulatory T cells (CD4+ CD25hi CD127-) previously sorted, 10,000 cells were cultured with IL-2 (300 U/mL) and Human T activator CD3/CD28 Dynabeads in a 1 bead:2 cells ratio in 200 µL of the previously indicated medium, refreshing it with 50 U/mL 3 days into the experiment. To analyze FoxP3 expression stability in the expanded cells, CD3 and CD28 stimulation with Dynabeads were removed from the culture on day 5. At 4 and 7 days of culture, iTreg conversions and Treg expansions respectively were analyzed by flow cytometry based on CD25 and FoxP3 co-expression.

Result: Nanoparticle tracking analysis (NTA) indicated adipocytes isolated from breast cancer associated adipose tissue from patients produce a significant amount of adiposomes (~1.07x109 particles/mL) with a size range of 100–200 nm, which varied between samples obtained from patients with different molecular subtypes of BC. By TEM we observed that adiposomes have a defined morphology and structure that is similar to those of other exosomes. Interestingly, the sizes of the exosomes from one patient to another changed and heterogeneity needs to be further explored and correlated with clinical histopathology.

In ex-vivo Treg induction experiments, 40–50% of CD4+ CD25+ FoxP3+ T cells were generated from CD25- CD45RA+ T cells after 4 days of culture. Preliminary results show that we are also able to expand Tregs from as few as 10,000 Treg cells ex vivo per well with a 50–60% of CD25+ FOXP3+ after 7 days.

A previous work on our group had stablished an exosome to cell ratio between 500:1 to 10000:1 which allows an enhanced conversion and expansion, thus the amount of adiposomes obtained from our patient samples are sufficient to analyze their effect on Tregs.

Conclusions**:** Purified adiposomes produced by BC associated adipose tissue, have the size and typical features of exosomes but they have differences in particle size depending on the type of BC patient of origin.

Our ex vivo experiments of Treg conversion and expansion indicate that it is possible to induce and expand Treg cells from breast cancer patients. We are currently investigating the effect of autologous adiposomes in Treg expansion and iTreg conversion as well as on Treg stability and function, to evaluate the effect of these vesicles in the pathology of cancer and data obtained could be correlated with clinical information.

Metabolic inhibition of tumoral anabolism and host catabolism in colorectal cancer

Adrián Alejandro Schcolnik Cabrera[1], Alma Chávez-Blanco[1], Guadalupe Domínguez-Gómez[1], Mandy Juárez[1], Donna Lai[2], Sheng Hua[2], Lucia Taja-Chayeb[1], Alfonso Dueñas-González[3]

[1]Instituto Nacional de Cancerología (INCan), Mexico, Mexico
[2]Bosch Institute, University of Sydney, Sydney, Australia
[3]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
alex.schc@gmail.com; celular_alma@hotmail.com; dguadalupeisabel@yahoo.com.mx; mandy.juarezr@gmail.com; donna.lai@bosch.org.au; sheng.hua@sydney.edu.au; ltaja_chayeb@yahoo.com; alfonso_duenasg@yahoo.com

Keywords: Colorectal Neoplasms, Cancer, metabolism and cancer, cell cycle regulation, Drug Synergism

The malignant metabolic phenotype is characterized by tumor anabolism and tumor-induced host catabolism, which is mediated by inflammatory molecules acting systemically. The main primary fuel for tumor anabolism are glucose, glutamine and fatty-acids. Hence, malignant tumors exhibit high rates of glycolysis, glutaminolysis and de novo synthesis of fatty acids. Colorectal cancer is highly prevalent and a number of metabolic dysregulations in both the host and the tumor itself have been reported elsewhere.

In this work we use a therapeutic repositioning strategy with drugs aimed to impede the tumour anabolism and decrease the host catabolism using human and murine colon carcinoma cells SW480 and CT26.WT, respectively. We use a combination of lonidamine, DON and orlistat (anti-glycolytic, anti-glutaminolytic and an inhibitor of fatty acid synthesis, respectively) to inhibit tumor anabolism, while indomethacin, insulin and growth hormone act as anti-catabolic drugs to inhibit tumor-induced host catabolism.

We have demonstrated the feasibility of the combination containing the three drugs against the tumor anabolism, which synergistically reduce cellular viability in both cell lines, while the use of growth hormone, insulin and indomethacin in combination do not alter cell viability. Cell cycle and apoptosis evaluations revealed that the combination of the anti-tumor anabolism drugs induce a strong blockade at G0/G1 cell cycle phase, and both early (CT26.WT) or late (SW480) apoptosis. siRNA blockades of the anti-tumor anabolic targets mimic the cell effects seen with the drugs. By using in vitro assays of cell metabolism we observed a blunt decrease in mitochondrial energetic function and in the glycolytic capacity with the use of lonidamine, DON

and orlistat, and not changes with the combination of inhibitors of the host catabolism. In vivo analysis demonstrated a similar reduction in tumour size with either the combination of the three anti-tumor anabolism drugs or with the six drugs together. Interestingly, the combination of drugs to block host catabolism did induce moderate tumor growth inhibition in vivo, which suggests that such combination may provide extra energy for the immune system to detect and attack the cancer. Currently we are evaluating the effect of the drug combinations upon serum cytokines in mice models at different times to analyze the progression of the immune response, and also we are analyzing diverse tissues including fat, muscle and tumor, to identify the pathological consequences of in vivo administration of all the drugs over time.

Role of the CCR9 chemokine receptor in the recruitment of FoxP3+ Tregs cells in the tumor microenvironment in murine colorectal cancer

Jacobo Martínez Ríos[1], Gloria Soldevila Melgarejo[2], Eduardo García Zepeda[2], Gisela DuPont Lara[2]

[1]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

[2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico belerofontej@hotmail.com; soldevi@servidor.unam.mx; garciaze@servidor.unam.mx; gisela@biomedicas.unam.mx

Keywords: Tumor Microenvironment, Colon Cancer, natural Tregs, CCR9, Tregs (or regulatory T cells)

Regulatory T cells (Tregs) are a specialized CD4+ T cell subpopulation that restrains immune responses and maintains immunological tolerance. They are characterized by the expression of Fopx3 transcription factor. FoxP3+ Tregs are essential suppressors of anti-tumoral responses and it has been shown that infiltration of these cells within the tumor microenvironment is significantly associated with poor prognosis in the majority of solid tumors. Interestingly, the infiltration of FoxP3+ Tregs in colorectal, head and neck, and oesophageal cancers is associated with better prognosis; however, other studies showed a poor prognosis, so the role of Tregs in colorectal cancer remains controversial.

Chemokines are chemotactic cytokines involved in leukocyte migration in homeostasis and in inflammatory conditions. Many cancers have its own chemokine network to attract immune cells with specific phenotype, among them Tregs cells. Tumor-infiltrating Tregs cells have specific chemokine receptor expression patterns compared to not only to other CD4+ T cell subsets, but also to Treg cells from normal tissues. Recently, it has been shown that CCR8 is specifically and highly expressed in tumor infiltrating Treg cells in colorectal and breast cancers, and its expression correlates with poor prognosis. However, other chemokine receptors could be also involved in the recruitment of tumor infiltrating Tregs cells. It has been reported that colon inflammation favors the development and progression of colon cancer. CCR9/CCL25 axis is responsible for the recruitment of immune cells to inflammed colon. However, the contribution of the chemokine receptor CCR9 to FOXP3 Treg recruitment to colon tumors has not yet been studied. To analyze the contribution of the CCR9 chemokine receptor in the recruitment of intratumoral Tregs cells and its impact on tumor devel-

opment, we developed a murine colon cancer model induced by AOM/DSS in CCR9 knock-out (KO) and heterozygous (HT) Balb/c mouse strains. In the murine colon cancer model induced by AOM plus DSS, all mice treated presented tumor development and tumors showed characteristics of well-differentiated adenocarcinoma. As a control and as previously reported, DSS alone treated mice, resulted inflammation in the absence of cancer development.

When Tregs were analyzed we found that there is an increase percentage of CD4+ CD25+ Foxp3+ Tregs in DSS groups compared to controls groups, but there were no significantly differences between HT and KO mice. In AOM/DSS groups there is a further increase in the percentages of peri-tumoral and tumor infiltrating Tregs in both HT and KO mice. Interestingly, we did not find significant differences between tumor infiltrating Tregs in KO mice compared to HT, but interestingly the percentage of peri-tumoral Tregs was significantly reduced in KO mice compared to HT. To determine if CCR9 affected differentially the recruitment of natural versus induced Tregs, we analyzed the expression of Helios, a transcription factor that identifies natural Tregs, in our model. The results showed that in DSS groups and in the peri-tumoral area of AOM/DSS groups, there is a reduced frequency of Tregs Helios+ in KO mice.

These results suggest that the chemokine receptor CCR9 modulates the migration of Tregs cells to inflammatory sites but not to the cancer microenvironment, consistently with the role of CCR9 in homing to the inflammed colon. Moreover, our data also indicate that natural Tregs are preferentially recruited via CCR9 to the colon under inflammatory conditions. In contrast, intratumoral Tregs appear to require other chemokine receptors to infiltrate the tumor. In this context it has been recently reported that CCR8+ intratumoral Tregs in colorectal cancer patients are enriched within the tumor, showing a more suppressive phenotype. Further studies are required to identify the CCR9 independent mechanisms responsible to recruit Tregs in the tumor microenvironment in colon cancer, that might be potential targets for clinical intervention.

Super-selective intraarterial chemotherapy in the primary management of diffuse intrinsic pontine glioma: 2-year experience from an institution in México

Alberto García, Alberto Siller, Paola García, Sixto Trejo

Instituto de Oncología Intervencionista (IDOI), Monterrey, Mexico
agfuente@hotmail.com; albertosiller@hotmail.com; info@idoimexico.com; Med.contact@idoimexico.com

Keywords: chemotherapy, pediatric, oncology clinical trials, dipg, SSIAC

Introduction: Diffuse Intrinsic Pontine Glioma (DIPG) is an aggressive tumor in the pons with a median overall survival of 9 months, ≥90% of patients won't survive 2 years after diagnosis.

Super-Selective Intraarterial Chemotherapy (SSIAC) is a delivery method that allows higher drug concentrations at the tumor site while exhibiting only mild systemic toxicity due to relatively low levels of the agent in circulating blood.

Aims: The presented study focuses on our experience treating patients diagnosed with DIPG using SSIAC with a non-protocolized, individualized set of Chemotherapeutic drugs.

Experimental strategies: Eligible subjects are under 18 years of age, with a radiological diagnosis or proven DIPG biopsy, excluding those whose tumor extends beyond the pons or with the presence of metastasis.

Initial evaluation and follow-up consist of MRI, PET/CT and Lansky play-performance score.

Results: From March2016 to April2018, 62 patient's clinical and radiological conditions were evaluated and treated by our team with intraarterial chemotherapy. A median of 7 intraarterial procedures per patient were performed. With age ranges between 2 and 17 years, 8 years on average, 22 male and 40 female, with a median of survival after the diagnosis of 489 days and counting.

The follow-up range for these results has a median of 6 months. A median of Lansky score 60% before treatments and a median of 80% after treatments.

45 are with stable disease, none with progression of the disease and 2 of them in remission, all presenting response to treatment. 17 deaths related to treatments prior to our intervention.

Immunological parameters were taken into account to perform the treatments.

Conclusions**:** Given the inconsistent efficacy of radiotherapy and the limitations of systemic chemotherapy, innovation in adaptable therapeutic methods is a necessity.

Patients with DIPG can benefit from being treated with SSIAC, resulting in radiological improvement and better quality of life.

Leukodepletion of donor platelet concentrates reduces TH17 cell differentiation and cytokine production on allogenic CD4+ T cells

Carolina J Hernández[1], Jaxaira Maggi[1], Paulina García-González[1], Josefina Barrera[1], Octavio Alexis Aravena[1], Diego Catalán Martina[1], Milton Larrondo[2], Juan C Aguillon[1]

[1]*Universidad de Chile, Santiago, Chile*
[2]*Hospital Clínico de la Universidad de Chile, Santiago, Chile*
cjhernandez@uchile.cl; jaxaira.maggi@gmail.com; paugarciag@gmail.com; josefinabarrera@gmail.com; aravena.octavio@gmail.com; diegocatalanm@gmail.com; mlarrondo@hcuch.cl; jaguillo@med.uchile.cl

Keywords: Th17 Cells, Immune Modulation, T CD4+ cells, Platelet concentrates, leukopletion of platelet concentrates

Allogeneic transfusions of blood components are medical procedures widely performed and validated as therapy. Nevertheless, in some cases, recipients of blood component transfusions present a state of transient immunomodulation called "transfusion-related immunomodulation" (TRIM) The immunological mechanisms leading to TRIM are not fully understood, however, some authors had related the exposure to allogenic mononuclear cells, as well as soluble mediators derived from leukocytes, to the modulation of the recipient immune response. Platelet concentrates are one of the most used blood components for therapy, however, since they are prepared from the leuco-platelet layer of blood, they can contain higher levels of leukocytes with no therapeutic purpose, and this can lead to the activation of non-desired allogeneic immune responses in the transfusion recipient. Our aim was to assess whether platelet concentrates activate CD4+ T cells in vitro and to determine if leukodepletion of platelet concentrates could reduce this effect on CD4+ T cells from healthy donors. For this purpose, platelet concentrates were obtained from ten blood donors at Hospital Clínico de la Universidad de Chile blood bank. A portion from the platelet concentrate was filtrated to obtained leukodepleted platelet concentrates and the rest of the concentrate was left untreated. Both products were stored in agitation at room temperature. After three days of storage, treated and untreated platelets were co cultivated with allogenic CD4+ T lymphocytes, previously purified by negative selection. CD4+ T cells were activated in vitro with anti CD3 and anti CD28 and then cultivated with platelets from regular concentrates or leukodepleted platelet concentrates for three days. Platelet-induced activation and proliferation of CD4+ T lymphocytes were evaluated by flow cytometry through CD25 surface expression and analysis of CFSE dilution. CD4+ T cell profiles TH1, TH2, TH17 and Regulatory were identified through lineage markers by flow cytometry and

cytokine secretion through ELISA from culture supernatants. Additionally, platelets were also identified with CD41 and platelet activation was evaluated by CD62P surface marker expression. Platelet concentrates induced activation and proliferation of CD4+ T cells. Leukodepletion of platelet concentrates showed a tendency to reduce TH17 cell differentiation and, also, IFN gamma and IL17 production ($p=0.0371$ and $p=0.0325$ respectively, Mann- Whitney test) when compared to regular platelet concentrates. We did not observe any effect on the CD4+ T cell regulatory population. These results suggest that leukodepletion of platelet concentrates could help reduce its effect over an allogenic immune modulation and further support the use of leukodepleted components in immunodeficient patients and opens the field for new studies in immunohematology and immunotherapies.

Treatment with the dopaminergic agonist Pramipexole induces changes in the peripheral immune response in Parkinson disease patients

Laura Virginia Adalid-Peralta[1], Diana Denisse Álvarez-Luquín[1], Asiel Arce-Sillas[1], Jaquelin Leyva-Hernández[1], Esteban Montes-Moratilla[1], Viridiana Vivas-Almazán[1], Citzielli Pérez-Correa[1], Ulises Rodríguez Ortiz[1], Catherin Boll[1], Gladis Fragoso[2], Edda Sciutto[2]

[1]*Instituto Nacional de Neurología y Neurocirugía (INNN), Mexico City, Mexico*
[2]*Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico*
adalid.laura@yahoo.com; danliuc@hotmail.com; dobleas_2005@hotmail.com; jackley91@hotmail.com; esteban22ntdv@hotmail.com; viridianaamane@gmail.com; citziaseret@hotmail.com; ulrdz@hotmail.com; bollneur@gmail.com; gladis@biomedicas.unam.mx; argentina54@hotmail.com

Keywords: Treg cells, Post-treatment, Monocites, Parkinson's diseased patients, without treatment

Introduction: Parkinson disease (PD), the second most frequent neurodegenerative disease, results from the death of dopaminergic neurons in the substantia nigra. PD progression has been associated to neuroinflammation. At a peripheral level, effector and regulatory cell profiles are altered, as well as cytokine levels. However, to date it is not known whether these alterations are related to treatment.

Objective: To analyze post-treatment changes in the peripheral immune response in PD patients.

Material and Methods: Nine previously untreated PD patients were recruited. A peripheral blood sample was obtained at the inclusion time, and a new sample was taken one year after treatment. Cell phenotypes were characterized by flow cytometry. Treg cells, regulatory CD8 cells, B regulatory cells, activated and tolerogenic dendritic cells; classical, intermediate, and non-classical monocytes; and Th1, Th2, and Th17 cells were analyzed. The patients were assessed according to the UPDRS and H&Y scales and treated with dopaminergic agonists, either alone or combined with levodopa.

Results: An increase in active Tregs, IL-10-producing intermediate monocytes, HLA-DR-expressing non-classical monocytes, and IL-17-producing Th1 and Th17 cells after

one year of treatment. A decrease in IL-13-producing Th2 cells was also observed one year after treatment.

Discussion and Conclusions: Our results showed an increase in Th1 and Th17 profiles, indicating an inflammatory environment in PD patients. An increase in the levels of active Tregs and intermediate monocytes was observed one year later, suggesting that the treatment induces a recovery in the regulatory immune response. A decrease of Treg levels has been described in PD, and this study demonstrated that after one year, the treatment favors a regulatory immune response, which coincides with a decrease in the scores of the UPDRS and H&Y scales.

The role of CXCL10 in BALB/C mice supplemented with zinc

Ana Esther Aguilar, Omar Martínez, Nelly Rodríguez, Margarita Rojas, Guadalupe Elizabet Orive

Faculty of Chemistry, National Autonomous University of Mexico, Mexico City, Mexico
dranitae@gmail.com; omarfacquim@gmail.com; nelly.r.di@gmail.com; magosajor@hotmail.com; anitae@unam.mx

Keywords: Inflammation, Zinc, nutrition, CXCL10, Th17

Introduction: CXCL10 is associated with a variety of human diseases including infectious diseases, chronic inflammation, immune dysfunction, tumor development, metastasis and dissemination. The chemokine CXCL10 is a 10 kDa protein induced by IFN-γ and was formerly known as IP-10. It is mainly secreted by monocytes and macrophages in response to stimulation with IFN-γ, LPS and TNF-α (1). The role of zinc and its effects on immune cells have been described. Because Zn deficiency is closely link to insufficient dietary intake, especially people from developing countries, elderly, vegetarian/vegans and patients with chronic diseases are affected. Zn supplementation can either restore or even improve immune function (2). This study deals with the effect of zinc concentration over CXCL10 production in mice.

Material and Methods

Experimental design

We used an experimental model of zinc supplemented mice (500 mg/L zinc acetate, BALB/c inbred mice). These animals were kept in plastic boxes covered with stainless steel and sterile bottom and they were fed ad libitum with specially formulated commercial feed (PRO-LAB® RMH 2500 5P14, USA).

Zinc supplementation

The mice received zinc acetate (500 mg/L) in drinking water administered from the day of mating throughout gestation, lactation and postweaning. The pups were supplemented during 6 weeks or 9 weeks. Mice are divided into 8 groups according to LPS stimulation and to Zn administration.

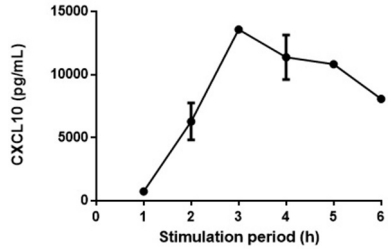


FIGURE 1: LPS stimulation provoked a significant increase in the CXCL10 production in BALB/c mice at 3 hours (Δ=95%)

LPS stimulation

Kinetic of production of CXCL10 was performed with bacterial lipopolysaccharide (Escherichia coli O55: B5, SIGMA CHEM L-2880, St. Louis, MO, USA) to cause acute inflammation, at a dose of 1 mg/kg, 200 microL (Fig 1).

Enzime Immuno Assay

CXCL10 was determinated in serum by an immunoenzymatic assay. Antibodies (PeproTech 900-M153) were employed to assess CXCL10 after mice LPS inoculation. Briefly, 96 well flat-bottomed microtritation plates coated with anti-CXCL10 polyclonal antibody (anti-CXCL10) were used. CXCL10 from the samples was captured in the wells, by the polyclonal antibody. A polyclonal biotinylated antibody anti CXCL10, was added and linked to the CXCL10 present in the solid phase, after incubation and washings. The reaction was made visible by adding avidine-peroxidase that bonded to the biotine; this in turn reacted with its substrate, H2O2 + ABTS, whose absorbance was read at 405 nm in a Behring EL microstrip reader.

Results and Discussion: CXCL10 has multiple roles, such as modulating innate and adaptive immune response. Our results show that zinc intervention during perinatal periods, produces an increment in secretion of CXCL10 (Fig 2).

Results during 6 weeks were that the group Zn500-6w showed 870 pg/mL of CXCL10. LPS controls induce 12200 pg/mL during 6 weeks of treatment meanwhile CXCL10 production was 670 pg/mL in the Zn0-6w group.

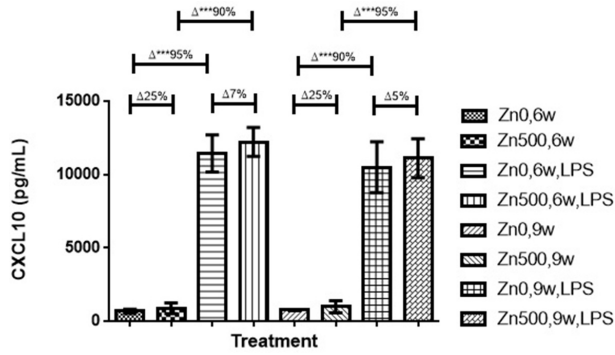


FIGURE 2: Zinc supplementation induced an increase (25%) in the CXCL10 concentration in BALB/c AnN mice Zn500-6w and Zn500-9w groups. Results were showed as the media ± standard deviation, chemokine pg/mL, n=40, data were analyzed by GradPhad Prism 7.03. *Statical significance ($p<0.05$)

During 9 weeks of treatment the zinc induction produced 970 pg/mL, compared with 750 pg/mL in Zn0 group. Finally the control with LPS was 11100 pg/mL.

One of the findings in this study is the regulation that provoke the zinc administration on the production of immunological components, particularly over chemokines. A disrupted zinc homeostasis causes impaired immune function leading to compromised host defense and increased risk of excessive inflammation.

On the other hand, in previous results we have showed that zinc supplementation modified IL-17 level. Th17 cells induced the recruitment of neutrophil granulocytes then zinc intervention could regulate chemokines production (3).

Conclusion: We conclude that supplementation of zinc in mice has the effect of causing increased production of CXCL10, which far to produced inflammatory problems this means a regulatory response to supplemented individuals. These results suggest the potential benefits of zinc intervention to prevention some autoimmunity disorders.

REFERENCES

1. Lui M, Guo S, Stiles JK. The emerging role of CXCL10 in cancer (Review). Oncol Lett. 2011, 2: 583-589
2. Maares M, Haase H. Zinc and immunity: An essential interrelation. Arch Biochem Biophys. 2016, 611, 58-65
3. Lastra A, Saldívar L, Martínez K, Munguia N, Marquez C, Aguilar AE, 2005. Zinc concentrations in mice during gestation, Biological Trace Element Research Vol 104 pp 1-11

Differential cytokine profiles according to inflammation level and steatosis in non-alcoholic fatty liver disease

Lorena del Rocío Ibarra Reynoso[1], Mara Anaís Llamas-Covarrubias[2], Benjamin Jordán Perez[3], Serafin Garnelo Cabañas[3], Juana Rosalba Garcia Ramirez[3], Monica del Carmen Preciado Puga[1], Alan Joel Ruiz Padilla[1], Maria Luisa Lazo de la Vega Monroy[1], Yeniley Ruiz Noa[1]

[1]*Universidad de Guanajuato, Guanajuato, Mexico*
[2]*Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico*
[3]*Hospital General de León, León, Mexico*
rey221280@gmail.com; mara.llamas@academicos.udg.mx; benjajordan1@gmail.com; seragarnelo@hotmail.com; rosy_gr@hotmail.com; mdc.preciadopuga@ugto.mx; alan.ruiz@ugto.mx; marialuisaqfb@yahoo.com.mx; yeni.rn@hotmail.com

Keywords: Inflammation, Liver Diseases, steatosis, NAFLD, cytokine profile

Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) houses a spectrum of lesions from simple steatosis to steatohepatitis, leading to hepatic fibrosis, cirrhosis, hepatocellular carcinoma and hepatic failure. Steatosis sensitizes hepatocytes to harmful mechanisms, which trigger an inflammatory response where cytokines are main orchestrators of disease progression.

Aim: To analyze Th1, Th2 and Th17 cytokine profiles according to inflammation level and steatosis in sera of NAFLD patients.

Materials and methods: 72 NAFLD patients were recruited and classified in 6 groups according to components of NAS activity score as follows: Group 1: Inflammation 0, steatosis 0; Group 2: Inflammation 0, steatosis 1-3; Group 3: Inflammation 1, steatosis 0; Group 4: Inflammation 2 - 3, steatosis 0; Group 5: Inflammation 1, steatosis 1-3; Group 6: Inflammation 2 - 3, steatosis 1-3. IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17 cytokines were measured by flow cytometry with the CBA Human Th1/Th2/Th17 BD™ Kit.

Results: We observed a significant difference on IL-4 levels among groups classified according to inflammation level and steatosis ($p<0.0006$) and a negative association between IL-4 and NAFLD activity index ($p<0.018$). Finally, no differences were observed on IL-2, IL-6, IL-10, TNF-α, IFN-γ and IL-17 levels among study groups.

Discussion and Conclusions: Our results show a role of IL-4 on NAFLD, were the immune response and the increased flow of free fatty acids to hepatocytes promotes the antigenic recognition of lipids. This, triggers a local immune response characterized by macrophage infiltration and polarization towards an M1 profile, as well as Th1 lymphocyte activation and cytokine secretion. The close regulation of this process through the activation of a number of cells such as: Th2 immunoregulatory lymphocytes, Kupffer cells, dendritic cells and endothelial cells; all of whom can secrete IL-4 and other cytokines, will determine if steatosis and inflammation is established or not, by promoting an increased activity of NAFLD.

Study of the cross-reaction antibodies of patients with dengue against ZIKA

Julio García Cordero[1], Mayra Montecillo Aguado[1], Alfredo Eduardo Montes Gomez[1], Hector Vivanco Cid[2], Leticia Cedillo-Barron[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico

[2]Instituto de Investigaciones Médico-Biológicas, Universidad Veracruzana, Veracruz, Mexico

leebeydengue@yahoo.com.mx; mayramontecillo@hotmail.es; freddym14@hotmail.com; hvivanco@uv.mx; lcedillo@cinvestav.mx

Keywords: Antibodies, Dengue Virus, E protein, cross-reaction, Zika virus

Introduction: Zika virus (ZIKV) is an arthropod-transmitted, positive sense RNA virus that is closely related to viruses causing human disease, such as dengue (DENV), yellow fever (YFV), West Nile (WNV) and Japanese encephalitis (JEV) viruses. Historically ZIKV infection in human has been associated with a self-limiting mild, febrile illness. Since its epidemic emergence in 2007, ZIKV infection has become linked to more severe clinical syndromes. The infection of pregnant women, particularly during the first trimester, can result in congenital Zika syndrome, which includes microcephaly, neurodevelopmental abnormalities and fetal demise In adults, ZIKV infection is associated with Guillain Barré syndrome (GBS). An autoinmune disease characterized by ascending paralysis and polyneuropathy. (1)

The ZIKV genome is a single open reading frame that encoding for a polyprotein that is processed to yield three structural (Capsid), pre, membrane/membrane (prM/M), and envelope (E) and seven non structural (NS) proteins. The ZIKV E protein is composed of three structural and functional domains. Domain II,(DII) containing a hydrophobic fusión loop (FL) epitope at the distal end. Further in domine III (DIII) it contain an immunnoglobulin like segment implicated in receptor binding and entry. The E protein is also the primary target for neutralizing antibody responses. (2)

ZIKV strains are classified into two genetics linages, the African and the Asian American linages, thus, there is a single serotype because their neutralization by serum and monoclonal antibodies (mAbs) is quite similar, ZIKV. Furthermore genetic clustering places ZIKV in close relationship to DENV, within the E protein showing 54–59% amino acid identity.(3)

This high identity leads to a high cross-reactivity that could result in the phenomenon of antibody dependent enhanced (ADE) as presented in DENV. Despite these evidences, there is no systematic study analyzing the cross reaction against ZIKV from patients with DENV disease in endemic áreas, before the ZIKAV was introduced.

Objective: To evaluate the cross-reaction against ZIKV from patients with different forms of acute dengue disease, and with any ZIKAV antecedents

Result: To obtain sufficient recombinat E-ZIKAV protein to be used as antigen in the home made ELISA assays. The sequence of E protein was subcloned in the plasmid pMT to then be expressed in Drosophila melanogaster S2 cells expression system, The vector contain a leader sequence to ensure efficient secretion of 6His-tagged soluble recombinant Zika protein E. Initially, the insert was released from the construction pUC-E and then ligated in pMTA plasmid (4). Three positively transformed colonies were selected. The colonies were grown in LB individually and plasmids were isolated from them. The construction pMTA-E-ZIKAV were confirmed by double digestion with KpnI + XbaI. And the DNA sequencing showed that the E sequence is in a correct ORF.

The expression of recombinant protein drived by the metallothionein promoter was evaluated. S2 cells transfected with pMT-EZIKV were treated with brefeldin A and then evaluated its protein expression by immunofluorescence. Positives immunoreactivity were detected using both an specific anti V5 antibody and anti-PAN Flavivirus (4G2), confirming expression of Zika E protein. The supernatant was analysed by Western Blotting using anti His and for evaluate the cross-reactivity we use one monoclonal antibody that recognize E protein DENV. The observed bands were consistent with the predicted size (aproximately 55 kDa). To produce larger quantities of recombinant protein for subsequent assays, stable transfectant cells were established and expanded in culture and 12 days after induction were harvest.

To evaluate the amount of specific antibodies generated against E ZIKAV, serum samples collected on days 5–7 from the onset of fever from clinically and serologically confirmed DF and DHF patients in 2010 before the ZIV was introduced in Mexico. Negative controls were represented by negative serum to DENV infection from Endemic Zone and all them were analysed by an in house developed ELISA assay that used the recombinant protein as antigen. The plates were coated with (3 µg/ml for E-ZIKV). Different dilutions of each serum sample were tested for reactivity with the recombinant protein in triplicate. And we observed that there cross reactivity against E-ZIKAV protein even in the serum from EZ. In contrast no highly reactivity against ZIKA E protein was observed in serum samples from non endemic zones.

Discussion: The E protein is the most exposed and immunogenic antigen on the Surface of the ZIKV particle and also is the most conserved in Flavivirus. Here, we evaluated the utiity of recombinant E-ZIKAV for evaluate the cross reactivity from serum that never has been in contact with ZIKAV, but that suffer an acute DEN infection. Our results demostrate that DENV immune and serum samples from endemic reacted strongly with E-ZIKAV antigen.

This data demostrate the higly homology in terms of immunogenicity betwen the members of the flavivirus family. This fact may conditionate the sever dengue or enhance ZIKAV infection.

Conclusion: The E protein of ZIKAV is recognized by serum from DENV patients that never have been in contact with ZIKAV.

REFERENCES

1. Guillian-Barre Syndrome outbreak caused by ZIKA virus infection in French Polynesia. Lancet 2016, 387, 1531–1539. [CrossRef]
2. Zika virus:Isolations and serological specificity. Trans. R. Soc. Trop. Med. Hyg. 1952, 46, 509–520. [CrossRef]
3. Construct and expression of recombinant domains I/II of dengue virus 2 and its efficacy to evaluate immune response in endemic área: Possible use in prognosis.
4. DNA priming e and NS1 constructs—homologous proteins boosting immunization strategy to improve immune response against dengue in mice. Viral Immunol. 18 (4), 709–721.

Identification of inflammatory molecules in monocytes challenged with lipopolysaccharide (LPS) of children with metabolic syndrome (MS)

Juan Antonio Gonzalez-Rojas[1], Alberto Daniel Saucedo-Campos[2], Jeanet Serafin-López[1], Isabel Wong-Baeza[1], Ruben Lopez-Santiago[1], Octavio Rodriguez-Cortes[3], José Rafael Jimenez-Flores[2]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[2]Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico

[3]Escuela Superior de Medicina (IPN), Mexico City, Mexico

grja04@gmail.com; beto000@msn.com; jeaserafin@hotmail.com; isa_wb@yahoo.com.mx; rlopezsantiago@hotmail.com; octaviordzc@yahoo.com.mx; jrjf@unam.mx

Keywords: Insulin Resistance, Monocytes, proinflammatory cytokines, HOMA-IR, Metabolic syndrome in children, LPS stimulation

In the last 30 years there has been an increase in overweight or obese people and it is estimated that 60% of the world population is affected by this metabolic disorder. These reports also reveal that children are one of the most vulnerable groups in the age group of the population, because they are easy target of advertising stimuli, predispose to infections of greater severity, atopy, joint and skeletal repercussions associated with overweight or obesity. These disorders are a priority of study and have the greatest attention by different national and international organizations because, the usual evolution "child with overweight-obesity, will become an obese adult". The world morbidity and mortality statistics establish that diabetes mellitus 2, obesity, hypertension, atherosclerosis and ischemic heart disease are among the most prevalent. Most of the countries have modified their health policies to reduce overweight-obesity through different strategies. The loss of metabolic balance due to sedentary lifestyles with high

Table 1: Intervals of the HOMA-IR index. This table shows the cut-off points used to classify insulin resistance

Classification	HOMA-IR
Healthy	< 2.5
Insulin medium resistance (HOMA 2.5)	2.5 - <3.5
Insulin resistance (HOMA 3.5)	>3.5

and constant intake of carbohydrates and lipids, allows the over storage of energy in the form of triglycerides, stored in adipose tissue, over time this energy excess has been considered as the beginning of a series of metabolic alterations (insulin and lipids) that in medical practice are grouped as metabolic syndrome (MS), progressively interferes in the normal neuroimmunoendocrinological functions of the organism and progresses to the clinical entities indicated.

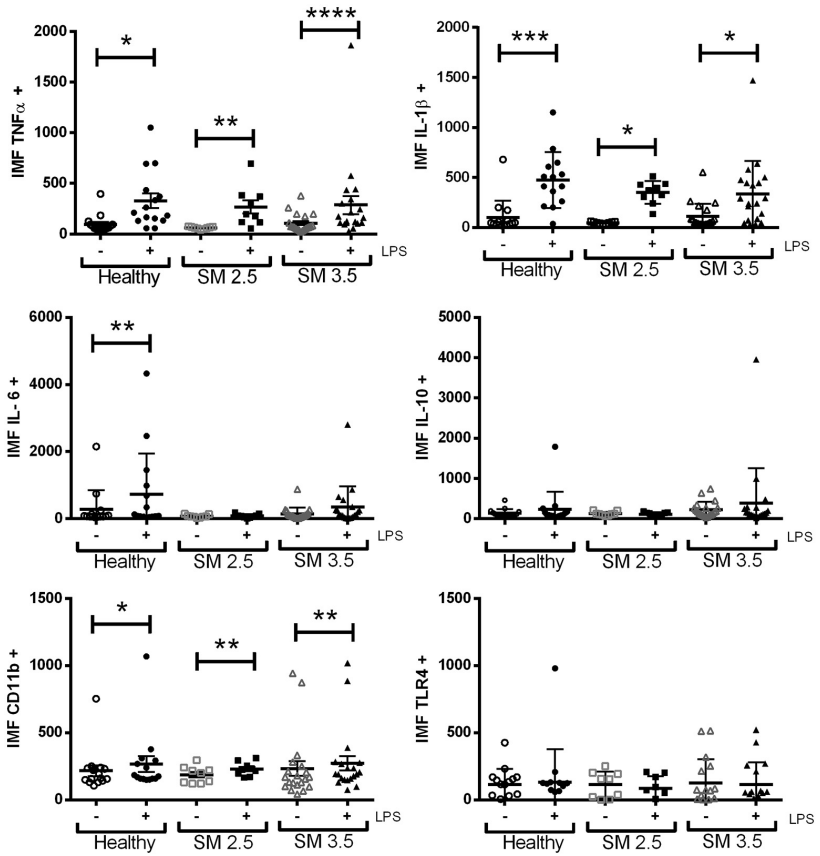


FIGURE 1: Percentage of cells positive for cytokines and surface molecules in monocytes. The different groups are observed: Healthy children, SM 2.5 and SM 3.5, as well as -LPS or + LPS. The results are shown as% of cytokine producing cells, Kruskal-Wallis statistical analysis, Dunn's multiple comparison * P <0.05, * P <0.01

The main alterations of the adipose tissue correspond to the energy imbalance, which is compensating through processes such as hyperplasia and hypertrophy, however when the amount of food does not allow to resolve the excess energy, in these conditions the adipose tissue undergoes various alterations in its functioning: from the synthesis of molecules that favor the reduction of intake (leptin) as well as regulating the synthesis of hormones such as insulin for the absorption and use of excessive carbohydrates in blood. If the lifestyle and eating habits persist, the alterations will increase, resulting in the establishment of an inflammatory microenvironment due to the release of fatty

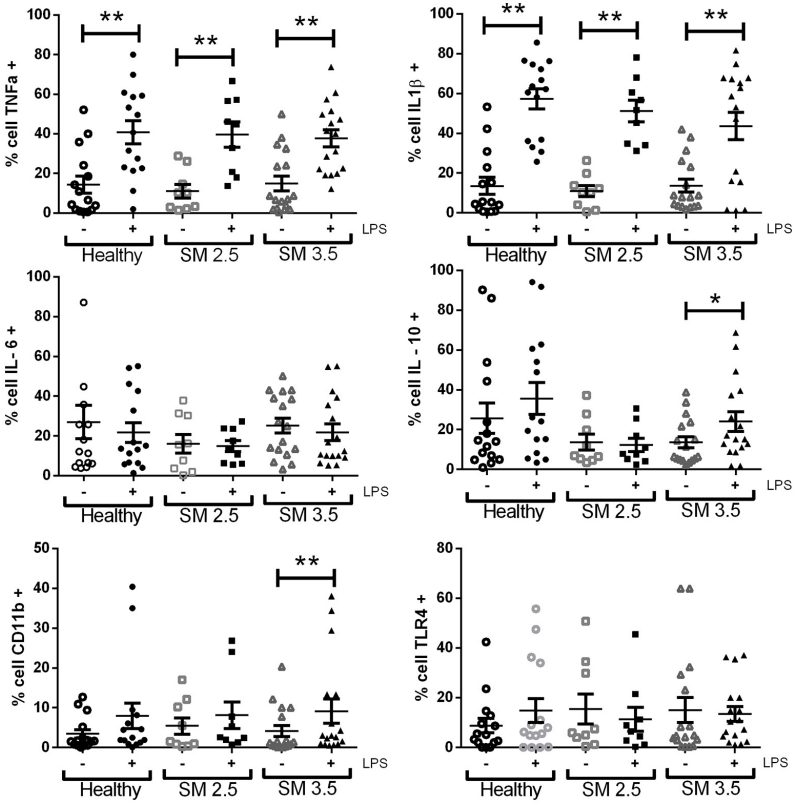


FIGURE 2: Production of cytokines and surface molecules in monocytes. The different groups are observed: Healthy children, SM 2.5 and SM 3.5, as well as -LPS or + LPS. The results are shown as MFI of cytokine producing cells, Kruskal-Wallis statistical analysis, Dunn's multiple comparison
* P <0.05, * P <0.01

acids and adipose tissue remains that die due to the low availability of oxygen. To face these conditions requires various mechanisms that can respond in this adversity environment. Some cells of the immune system are residents of the adipose tissue and when they detect an alteration in their environment, respond with an opportunity to give a prompt resolution. A biologically very active cell with a relevant participation in these processes is the monocyte. This cell acts in defense against microorganisms and gives rise to macrophages (M1, M2), which have been reported as cells that probably have a relevant participation in various metabolic alterations, because they are able to recognize alterations of the microenvironment through of receivers, among which are highlighted by their importance TLR4. This receptor has the capacity to recognize LPS, it also responds to elements such as free fatty acids and oxidized LDL, it is attributed the ability to participate in the synthesis of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, as well as increasing the expression of adhesion molecules (CD11b). It is important to note that these processes in individuals with obesity occur simultaneously and constantly, so that their altered but delicate balance allows the proper functioning of the organism without compromising other systems or functions, so they are painless and not perceived as Chronic inflamation.

In the present study 47 children were studied, 17 healthy and 30 with SM, (3 or more criteria). The most frequent altered parameters were: BMI, area of visceral fat, triglycerides and insulin resistance (table 1). From peripheral blood collected with heparin, 100 µl was placed in separate tubes. One of them (+) was stimulated ex vivo with 500 ng of LPS (Sigma-Aldrich MO, USA) was incubated for 5 hours, after the first hour was added 1 µl of Brefeldin A (BioLegend CA, USA), passed time was used antibodies against CD14-FITC, CD11b-PerCP, TLR4-PE (BioLegend) incubating for 20 minutes, then with lysis solution (BioLegend) at 4 ° C in the dark, washed and permeabilized before adding the intracellular antibodies : TNF-α-APC, IL-1β Alexa 647, IL-6-PECy7 and IL-10-PECy7 (BioLegend) were incubated for 20 minutes, fixed with 500µL of 1% PFA, analyzed in the FACSAria II .

The results show no statistically significant differences in the production of cytokines or in the percentage of cytokine-producing cells in children with MS compared with healthy children, (Figure 1 and 2) our results with the described strategy do not agree with other works published nevertheless, it can be concluded that in children with a clinical diagnosis of MS from 7 to 14 years of age, they have high BMI, triglycerides and insulin in 100% of the participants, curiously none of them has hyperglycemia.

Development of a lateral flow assay for the differential diagnosis of active and latent tuberculosis in Mexican population using serum biomarkers

Juan Jose Oropeza Valdez[1,2], Juan Ernesto Lopez[3], Julio Enrique Castañeda Delgado[1,4], Jose Antonio Enciso-Moreno[1]

[1]Unidad de Investigación Biomédica de Zacatecas (IMSS), Zacatecas, Mexico
[2]Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico
[3]Autonomous University of Aguascalientes, Aguascalientes, Mexico
[4]Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico City, Mexico
intrinsection@hotmail.com; ernesto.lopez92@hotmail.com; julioenrique_castaeda@yahoo.com.mx; enciso_2000@yahoo.com

Keywords: Latent Tuberculosis, biomarkers, Active tuberculosis, Tuberculosis DIagnosis, Lateral flow assay, Diagnoscti Test

Tuberculosis continues to be a worldwide health issue and is the first cause of death by an infectious agent. The current conventional diagnostic tests for active tuberculosis (ATB) have many limitations such as a lack of fast results in the case of culture and accuracy in the case of acid-fast smear test. It would be highly desirable to use new and more specific biomarkers that could define treatment efficacy, disease activity, cure and relapse. Therefore an affordable assay that includes biomarkers with application in resource-limited setting is urgent and necessary . Here we describe the development of lateral flow assay (LFA) for the differential diagnosis of active and latent tuberculosis using Biomarker 1 and Biomarker 2 peptide as biomarkers in serum. A colloidal gold LFA was designed to detect Biomarker 1 and Biomarker 2 using serum samples from 3 different groups: ATB, Latent tuberculosis infection (LTBI) and healthy controls (HC). The limit of detection for the Biomarker 1 LFA was 100 ng/ml. ATB and LTBI obtained a positive result at different time points and the HC group obtained a negative result in most of the cases. Statistical diagnostic analysis using ROC curves, showed that a sensitivity of 100 % (CI 95%: 96.67–100%) and a specificity of 96.67% (CI 95%: 88.58–100%) was reached using a cut-off point in time of 31.5 min for a ATB positive result. These results demonstrate the potential use of the Biomarker 1 LFA for ATB diagnosis. Further research is needed in order to validate the test in a larger group of samples.

High body fat percentage, physical inactivity, and male children's telomere length

Roberto C. Licea-Cejudo[1], Martha Pedraza-Escalona[1], Mónica V. Martínez-Martínez[2], Alfonso Carreón-Rodríguez[3], Gustavo Pedraza-Alva[1], Leonor Pérez-Martínez[1]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Instituto Nacional de Salud Pública, Cuernavaca, Mexico
robelice@ibt.unam.mx; mapedmx@yahoo.com; monviviana31@hotmail.com; diamanteajedrez@gmail.com; gustavo@ibt.unam.mx; leonor@ibt.unam.mx

Keywords: Inflammation, Obesity, Ageing, telomeres, physical inactivity., obese children, Body-Mass Index, Body fat percentage

The prevalence of childhood obesity in Mexico is increasing rapidly, being today the country with the largest number of obese children worldwide1. Several studies have associated obesity with non-metabolic conditions and accelerated cellular processes similar to those of aging, such as telomere shortening2,3,4. Telomeres are specialized structures of DNA and proteins located at the ends of eukaryotic chromosomes and have a crucial role in maintaining the genome integrity5,6,7. Telomeres become progressively shorter with each cell division8, and telomere shortening has been shown to be exacerbated by oxidative stress9 and inflammation10, mainly due to the increased production of reactive oxygen species and the damage they cause to DNA11. Telomere shortening promotes cellular senescence12, a phenotype in which normal cells stop their proliferation irreversibly and acquire different morphological characteristics, as well as changes in gene expression13, which result in the deterioration of tissues, organs and loss of longevity, all these, characteristics of aging.

This study was aimed to determine if there is a direct relationship between telomere length and obesity in a group of Mexican children of school age. To answer this question, a qPCR protocol was optimized for measurement absolute telomere length. This protocol was validated in an in vitro model of cellular senescence using human embryonic kidney cells, in which the telomeric length decreases depending on the number of cell divisions and in response to reactive oxygen species. The absolute telomere length of 134 children of both sexes between 8 and 10 years of age was determined, of which 47% were diagnosed with obesity, 19% were overweight and 34% had a healthy weight. We found that male children with a high body fat percentage showed 29% shorter telomeres compared with their lean counterparts, whereas this phenomenon was not observed in females. In addition, we observed that physical activity exerts a protective

effect on the length of telomeres, since the absolute telomere length of children who do not perform physical activity was 20% shorter in comparison to those children who perform it. Taken together our data indicate that physical activity and a healthy percentage of fat exert a synergistic protective effect on the length of telomeres.

This work was partially supported by grants from DGAPA/UNAM (IN213316, IN212316 and IA203416) and CONACYT (2282).

CXCL16 and Zn influence over human nutrition

Ana Esther Aguilar, Omar Martínez, Margarita Rojas, Nadia Marcela Munguia

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
dranitae@gmail.com; omarfacquim@gmail.com; magosajor@hotmail.com; nadia.munguia@gmail.com

Keywords: Chemokines, Zinc, food intake, CXCL16, Nutritional Immunology

Introduction: Zinc plays important roles in several clinical and physiological functions (1). This micronutrient is part of the structure and function of enzymes and transcription factors. Food sources that contribute whit Zn are fish, seafood, red meat, huitlacoche and oysters (2).

Deficiency in the consumption of zinc and the low bioavailability of the element in some food, suggest the importance to carry out studies on the intake of zinc in the daily diet, as well as to provide data to reach the values in the Recommended Daily Intake, RDI, and thus to avoid the appearance of adverse effects on health (1, 4).

University students aged 22-24 years recorded their food and drink intake for five consecutively and completed 24-hour dietary recalls on three random during this 5-day study period. To know the amount of zinc contained in each of the foods or preparations Nutrikcal VO® software was used (3).

Previous results our laboratory have demonstrated that a disrupted Zn homeostasis to compromise host defense according with Prasad et al (1).

Objective: Our study investigated relation between the nutritional status of zinc and the immune response in university students by evaluating zinc intake, zinc concentrations in hair and the production of CXCL16.

Material and Methods

CXCL16 ELISA

Serum sample were obtained. Production of CXCL16 and its detection in serum was assayed a double antibody EIA (capture antibody and detection antibody, PeproTech, 900 M-230, USA), Where antibodies anti-CXCL16 were produced in rabbit

and the second antibody was biotinylated; as developing agent was employed the avidine-peroxidase system plus ABTS (Sigma, A3219) and absorbance was measured to 405 nm in a Behring EL 301 microreader, with correction at 650 nm.

24-hour dietary recalls

University students aged 22–24 years recorded their food and drink intake for five consecutively and completed 24-hour dietary recalls on three random during this 5-day study period. To know the amount of zinc contained in each of the foods or preparations Nutrikcal VO® software was used.

Zinc concentration in hair samples

Each of the participants donated a hair sample; samples were taken from the occipito-nape region within 3 cm of the hairline, mass approximately 50mg of pretreated hair. Dried hair sample were digested with 2mL suprapur HNO3 (Merck, Germany) and 1 ml of H2O2 30% were added (Merck, Germany). Each sample was analyzed by AAS.

Results and Discussion: Previous results of our laboratory have demonstrated that a disrupted Zn hemostasis to compromise host defense according with Prasad et al. We obtained median value of CXCL16 for students of the School of Chemistry, 250 pg/mL (Fig.1 and 3). The values of the concentrations of CXCL16 in healthy volunteers determined in a research group in Europe was around 1000 pg/mL. It is important to consider the bioavailability of zinc and its absorption.

Determination of zinc in hair and expression of CXCL16 showed a proportional ratio of 25% , as well as a 50% proportional relation between the concentration of CXCL16 in serum samples with the intake in the daily diet, however when analyzing the three parameters taken together is not clear (Fig. 2 and 4).

It is known the important role of zinc in both adaptive and innate immunity, including important role as a signalling molecule. For this, it is necessary further protocols, increasing the number of volunteers to be able to confirm the role of zinc over production of CXCL16.

Conclusion: Zinc induced the production CXCL16. It is expected that the consumpion of a dietary supplement can to improve the overall health and consequently improved cellular and humoral responses.

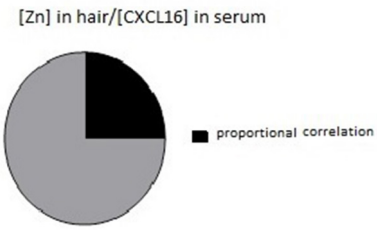


FIGURE 1: Correlation of the concentration in hair and the concentration of CXCL16 in serum. Principal finding was that 25% of people showed proportional correlation between Zn in hair and the expression of chemokine

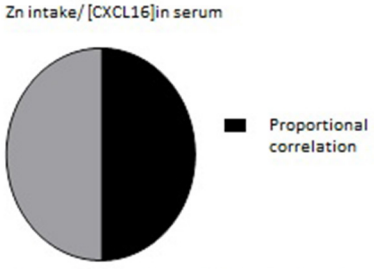


FIGURE 2: Mean Zn intake were calculated and CXCL16 serum concentration in serum (135 Zn mg/L /250 pg/mL CXCL16). 50% of the volunteer showed a proportional correlation between the amount of zinc in the diet and the concentration CXCL16

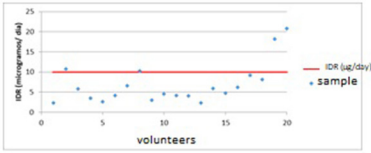


FIGURE 3: Intake of Zn in the daily diet and IDR of each of the volunteers. The figure shows that only 20% of the study population consumes what is recommended by the zinc IDR

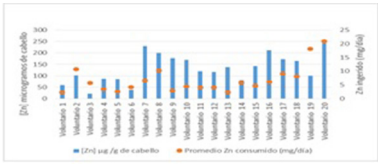


FIGURE 4: Daily consumption of Zn (mg/day) compared to the concentrations of Zn in hair (µg /g) of each of the volunteers

REFERENCES

1. Lastra A, Saldívar L, Martínez K, Munguia N, Marquez C, Aguilar AE, 2005. Zinc concentrations in mice during gestation, Biological Trace Element Research Vol 104 pp 1-11
2. Herrero E., Vigil A. (2003). Metodología recomendada para la medición del contenido de zinc en especímenes biológicos. Química clínica, 22, 13-18,Centro de Investigación en Nutrición y Salud Instituto Nacional de Salud Pública, Manual de procedimientos para proyectos de nutrición, México, 2006, pág.30
3. Food and Agriculture Organization of the United Nations, Nutrición humana en el mundo en desarrollo, Carencia de Zinc, 2002, 20, pág.205 Grases, F. , Simonet, B.M. , Perelló, J. , Costa, A. , & Prieto, R. (2004). Efffect of phytateon element biovailability in the second generation of rats. Trace Elem. Med. Biol. , 17, 229-234
4. Rubio C, Gonzalez D, Martin-Izquierdo RE, Revert C, Rodriguez I, Hardisson A, 2007.El zinc: oligoelemento esencial. Nutr Hosp. 22 (1): 101-107.

Extracellular vesicles isolated from septic patients enhance leukocyte transendothelial migration

Patricia Esther Miranda Cruz[1], Constantino López-Macías[2], Eduardo Antonio Ferat Osorio[2], Armando Isibasi[2], Eduardo Perez-Salazar[1], Lourdes Andrea Arriaga Pizano[2]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico

[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico

patriciae.mirandac@gmail.com; constantino@sminmunologia.mx; eduardoferat@prodigy.net.mx; isibasi@prodigy.net.mx; jperez@cell.cinvestav.mx; landapi@hotmail.com

Keywords: Exosomes, Sepsis, Transendothelial migration, Extracellular vesicles (EVs), Microvesicles (MVs)

Extracellular vesicles (EVs) and their cargo play an important role in cell to cell communication, they can be transferred between cells driving phenotypical and functional changes in the recipient cell. Several studies have reported an augmented number of EVs from circulating platelets, granulocytes and endothelial cells in blood of septic patients. Moreover, this increase in EVs has been associated with higher mortality rates of septic shock patients. It is well recognized that in patients with sepsis, leukocytes and endothelial cells increase their expression of adhesion molecules, chemokine's secretion and endothelial permeability. Recently, several studies have suggested that EVs isolated from macrophages stimulated with LPS contain adhesion molecules, pro-inflammatory cytokines and chemokines. However, so far the role of circulating EVs isolated form septic patients in leukocytes transendothelial migration remains to be studied. The aim of this study was to determine if EVs of patients with sepsis modify leukocytes transendothelial migration. Using an in-vitro model of transendothelial migration, consisting of Boyden's chambers covered with a monolayer of endothelial cells and after adding leukocytes in the upper chamber, leukocytes were recovered 2 h later from the lower chamber to evaluate leukocyte transmigration. Treatment (12 h previous to transendothelial migration assays) of either, endothelial cells or leukocytes, with EVs isolated from septic patients significantly increased leukocyte transendothelial migration. Although treatment of endothelial cells with EVs did not change PECAM-1, ICAM-1 and VCAM-1 expression, it induced endothelial cells migration in a wound-healing assay, a characteristic related with endothelial-mesenchymal transition. In conclusion, circulating EVs produced during sepsis will enhance leukocyte transendothelial migration by influencing both leukocytes activation and endothelial cells transition.

Aging affects frequency of non-classical monocytes but not other immune cell population in human

María de los Angeles Vázquez-Prieto, Nilda Belén Fernández-Eufrasio, Adriana Sumoza-Toledo

Universidad Veracruzana, Veracruz, Mexico
mariadelosangeles1vp@gmail.com; nilda18@hotmail.com; asumoza@uv.mx

Keywords: Aging, Inflammation, Monocytes, innate immunity, immunosenece

Introduction: Aging is characterized by the presence of chronic inflammation, a state called "inflamm-aging", in combination with a functional deterioration of the immune system, known as "immunosenescence". As a result of aging, responses to pathogens or vaccines are poor, increasing the risk of morbidity and mortality among the elderly(1,2). Data in the literature are inconsistent and even contradictory, for instance, changes in the frequency of the immunological cell subsets and the expression of their receptors are still controversial(3). Therefore, in this study, we evaluated the percentage of the subsets of immune cells in peripheral blood and the levels of PRRs and chemokine receptors in elderly subjects compared with young adults.

Material and Methods: Six healthy elderly subjects (60–80 years) and six young adults subjects (20–35 years) were examined in this study. Peripheral blood was collected in BD vacutainer tube containing EDTA as anticoagulant. Next, 50 µl blood aliquots were prepared in Eppendorf tubes and were blocked with purified anti-human CD32 (Biolegend), appropriate combinations of antibodies for immune cell subpopulations, pathogen recognition or chemokines receptors were added to them, and then incubated for 15 minutes under dark conditions. Red blood cells were lysed by adding BD FACS™ lysing solution to samples. Finally, samples were analyzed in the BD Accuri™ C6 flow cytometer. Data analysis was performed using Prims 6 (GraphPad Software). The Student's t-test was used to determine statistical significance ($p < 0.05$).

Results: There were no significant differences in most of the immune cell subsets examined in young and elderly subjects: neutrophils (81.04% and 85.70%), eosinophils (5.50% and 5.96%), intermediate monocytes (27.09% and 26.02%), classical monocytes (55.76% and 57.92%), B lymphocytes (22.95% and 21.60%), T lymphocytes (22.95% and 21.60%), T CD8 (50.83% and 51.43%), and T CD4 (53.73% and 53.83%). In contrast,

total monocytes (11.28% and 15.03%) and non-classical monocytes (0.45% and 2.5%) showed significant differences ($p = 0.0130$ and $p = 0.0108$, respectively). In turn, the expression level of TLR1, TRL2, TLR4, FPLR1, CXCR4, CXCR2, CCR2, CXCR7, and CD116 were not significantly different in both study groups.

Conclusions: Our data suggest that aging does not affect the frequency of the immune cell subsets, except for the total monocytes and its non-classical subpopulation, which were increased in elderly subjects; previously also reported(4). This monocyte subset has been suggested to be increased in adults with sepsis, systemic lupus erythematous and other conditions associated with increased inflammation(4,5). On the other hand, no significantly changes were observed in the levels of pathogen recognition or chemokines receptors, although we observed a tendency to less expression of TLR4 in monocytes from elderly subjects. It has been published that the activation of TLR4 with LPS in elderly is diminished(5), suggesting that the low response to pathogens is the reason for an increase of infections in this group. Therefore, we still need to compare the response to pathogens in young and elderly subjects.

REFERENCES

1. Alexander Panda, et. al. Human innate immunosenescence: causes and consequences of immunity in old age. Trends immunol. 2009 July: 30 (7): 325-333.
2. Shaw A. et. al. Aging of the innate immune system. Current Opinion in Immunology 2010, 22:507–513
3. Desai A. et. al. Leukocyte function in the aging immune system. Journal of LeukocyteBiology. Vol.87, No.6. 2016; 1001-1009
4. Ratnadeep M. et. al. Non-Classical monocytes display inflammatory features: Validation in sepsis and system lupus erythematous. Nature: Scientific reports. July 2015.
5. Thilinie Bandaranayake, Albert C. Shaw. Host Resistance and Immune Aging. Clin Geriatr Med 32 (2016) 415–432.

Comparison between the PPD and the detection of igm and IGG antibodies against M. Tuberculosis by the Elisa test as methods of diagnosis and follow up of latent and active TB in diabetic patients type 2

Alma Yolanda Arce Mendoza, Alberto Yairh Limón-Flores, Edgar Ivan Montes Zapata, Mayra A. Rodríguez-Serrato, Mario Cesar Salinas-Carmona

Facultad de Medicina, Universidad Autonoma de Nuevo León, Monterrey, Mexico
aya.mayola@yahoo.com; aylf10@hotmail.com; edivanmon@gmail.com; mayra_92_c@hotmail.com; mario.salinas@uanl.mx

Keywords: Diabetes Mellitus, Type 2, Tuberculin Test, Tuberculosis, Tuberculosis, Pulmonary, PPD

Introduction: There is currently an increase in cases between diabetes and tuberculosis (DM2 / Tb), which has caused great mortality in patients with this binomial throughout the world, which is why it is currently considered a major health problem. All in developing countries. Because of this it is considered very important to detect Mtb infection in time, therefore the need arises to find a diagnostic test that is rapid, sensitive and specific for the diagnosis mainly of latent infection and active tuberculosis. One of the serological tests is the one developed in our laboratory with a combination of extracellular proteins containing ESAT-6 and CFP-10 from Mtb with which the determination of IgM and IgG antibodies is carried out. It should be remembered that the IgM antibody is present in the serum until the antigenic stimulus disappears and declines over time, but not IgG antibodies that rise with the antigenic stimulus and remain for years as immunological memory.

Methodology: A total of 193 subjects divided into four groups were included: 1) 50 diabetic patients type 2 (DM2), 2) 46 patients with diabetes and pulmonary tuberculosis (DM2 / Tb), 3) 48 patients with pulmonary tuberculosis (Tb) and 4) 49 clinically healthy subjects. Each of the subjects studied was given informed consent, a survey was carried out to determine risk factors, and a peripheral venous blood sample was taken. Subsequently, the samples were analyzed using the patent ELISA technique 285260.

Results: When performing the statistical analysis, an average age was found in DM2 / TBP of 57 years, DM2 59 years, TBP 35 years and Healthy 32 years. The average glycemia was for DM2 / TBP 160mg / dl, DM2 144mg / dl, TBP 101mg / dl and Healthy

96mg / dl. It was found that around 63 to 70% of the subjects are not exposed to a patient with Tb and that around 13 to 20% of them spend more than 6 hours exposed to a patient with Tb. Regarding the results of the tests for the diagnosis of Tb, in the DM2 / TBP group 0% was negative for IgM / IgG, 52% tested positive for IgM, 48% positive for IgG, and 87% positive for PPD; in the DM2 group, 20% tested IgM / IgG negative, 48% tested positive for IgM, 56% tested positive for IgG and 32% positive for PPD; In the TBP group, 0% tested IgM / IgG negative, 63% tested positive for IgM, 100% tested positive for IgG and 63% tested positive for PPD; in the healthy group 44% were IgM / IgG negative, 2% IgM positive, 40% IgG positive and 43% positive PPD. The odds ratio was calculated which evaluates the possibility that tuberculosis is present in diabetics adding risk factors such as the evolution of the disease, glycemia and the time of exposure with Tb patients; this value increased from 2.22 to 15.04.

Discussion: In patients with tuberculosis an average age of 35 years is observed, the average time of evolution of diabetes was 11 years, similar to that reported by Durán Varela with an average of 13 years. When observing the results of IgM compared with the results of the PPD, the IgM determination was more sensitive to detect both a latent infection especially in diabetics not diagnosed with Tb, as well as the effectiveness of the treatment against Tb which can be observed in the groups diagnosed with Tb, where the IgM levels were already under treatment, the cellular response determined by the PPD test, which was still increased, was not decreased. In healthy individuals, the ELISA test was able to detect a subject with latent infection who manifested being exposed for more than 6 hours to a patient with Tb, this is of great importance to detect early infection in this type of subjects and take the necessary prophylactic measures to prevent the development of the disease. With the calculation of the odds ratio we were able to determine that daily contact greater than 6 hours, with a glycemia greater than 101 mg / dl and a time of evolution of DM2 greater than 5 years are risk factors with an increase of 15 times the risk of having a latent infection presenting a positive result for IgM in the ELISA serological test developed in our laboratory.

Conclusion: In the study we observed that the ELISA test is useful in the study of diabetic patients, especially when these are contacts (more than 6 hours a day) of someone with tuberculosis. The results allow to establish a prioritization of the medical attention of the contacts of a patient with tuberculosis, especially those who are diabetic, who present a blood glucose level that is out of control, with a time of evolution of diabetes greater than 5 years and very especially that present a daily contact with a patient with pulmonary tuberculosis greater than 6 hours a day.

Determination of IgA levels in saliva of patients with Systemic Lupus Erythematosus to assess SIgAD

Sandra Romero-Ramírez[1,2], Erick Saúl Sánchez-Salguero[3], José Jiram Torres-Ruiz[4], Diana Gómez-Martín[2,4], Rommel Chacón-Salinas[1], José Luis Maravillas-Montero[2]

[1]Instituto Politécnico Nacional, Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[3]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Ciudad de México, Mexico
[4]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
sandyyesorno@gmail.com; raziel_1218@hotmail.com; josetorresruiz85@gmail.com; d_gomar@hotmail.com; rommelchacons@yahoo.com.mx; maravillas@cic.unam.mx

Keywords: Saliva, IgA, ELISA, SLE, SIgAD

Introduction: Selective immunoglobulin A deficiency (SIgAD) is the most common primary antibody deficiency. The worldwide incidence of this disease varies depending on the ethnic background. SIgAD is diagnosed when IgA serum levels are less than 7 mg/dL in the presence of normal serum levels of IgG and IgM.

IgA is the main class of antibody present in the body-secreted fluids such as tears, intestinal mucous, calostrum and saliva. Unfortunately, immunoglobulins are typically measured in venous or capillary blood; however, alternative samples including saliva should be considered given its non-invasive nature and the easiness of its collection.

Patients with SIgAD are usually asymptomatic but they may suffer pulmonary infections, allergies, gastrointestinal disorders and, interestingly, a high frequency of autoimmune diseases when compared with the general population. It has been previously reported that SIgAD is frequently found in patients previously diagnosed with autoimmune diseases such as Systemic Lupus Erythematosus (SLE) (1–5.2%).

Aim: The aim of this study was to evaluate IgG, IgM, IgA1 and IgA2 concentrations in the serum and saliva of healthy individuals and SLE patients and to determine the frequency of SIgAD in patients with SLE. Salivary IgG, IgM, IgA1 and IgA2 levels were measured by enzyme-linked immunosorbent assay (ELISA) in 59 patients with SLE and they were compared with the immunoglobulin concentration in the saliva of 32 healthy volunteers.

Result: Currently, we have standardized the ELISA to measure the concentration of each immunoglobulin in serum and saliva samples. In healthy controls, we found that that the assessment of the salivary immunoglobulin concentration is possible and accurate with the procedures employed for collection and preservation. As we are still recruiting more individuals, both from a cohort of SLE patients and healthy controls, we are starting the analysis of salivary samples from lupus patients to be compared with the data available from the healthy control subjects.

Conclusion: The developed ELISA technique allowed us to efficiently determine the serum and specially, salivary immunoglobulin concentration in healthy controls and eventually, in SLE patients. Our validated assay will enable the screening and diagnosis of SIgAD among the cohort of SLE patients in the near future.

Up regulation of TYRO3 tyrosine kinase receptor and PROS1 in the myeloid compartment of patients with Langerhans cell Histiocytosis (LCH)

Cinthia Mariel Olexen[1], Wanda Nowak[2], Onias Castellanos Collazo[2], Juan Manuel Ortiz Wilczyñski[1], Licina Tessone[2], Ivana Gisele Estecho[2], Diego Alfredo Rosso[3], Andrea Emilse Errasti[2], Eugenio Antonio Carrera Silva[1]

[1]Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina
[2]Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina
[3]Hospital Pedro de Elizalde, Buenos Aires, Argentina
cinthia.olexen@gmail.com; wannowak@yahoo.com; oniasbio12@yahoo.com; juanma.ow@gmail.com; licitessone@gmail.com; ivanaestecho@gmail.com; drosso_2000@yahoo.com; andreaerrasti@gmail.com; carrerasilva@yahoo.com

Keywords: Myeloid Cells, langerhans cell histiocytosis, PROS1, Neoplasic, tyro3

Background: TYRO3, AXL and MERTK (TAM) tyrosine kinase receptors and its cognate agonist Protein S (PROS1) have been identified as negative regulators of the immune response, as well as non-classical proto-oncogenes aberrantly expressed in multiple haematological and epithelial malignancies. Langerhans Cell (LC) Histiocytosis (LCH) is a disorder characterized by an abnormal accumulation of CD207+CD1a+ myeloid cells in almost any tissue. The etiology of this disease is still under scientific discussion and it is not clear if LCH results from malignant transformation or unbalanced immune response that leads to the proliferation of pathogenic LC-like cells. For the current study, our main goal was to compare TAM axis profile in pediatric LCH patients with active (AD) vs non-active (NAD) disease.

Methods: We analyzed the expression of TAM receptors and PROS1 in peripheral blood mononuclear cells of pediatric patients with confirmed diagnosis of LCH, stratified as unisystem or multisystem with active disease or non-active disease and adult controls. The expression levels of PROS1 and TAM receptors were determined by flow cytometry and expressed as fold increase of mean fluorescence intensity (MFI) compared to the isotype control.

Results: Circulating total CD11b+ fraction was significantly expanded in AD (36.4 ± 3.7 % of positive cells N=11) vs NAD (18.9 ± 1.7 % N=12) and adult controls (24.9 ± 1.3 % N=12). Interestingly, this fraction that is considered the main source of inflammatory myeloid cells, showed significantly higher levels of PROS1 in AD (14.2-fold N=4) compared with NAD (6.0-fold N=5) and adult controls (6.7-fold N=6). TYRO3 was also up regulated in circulating CD11b+ cells in AD (10.6-fold N=8) compared with NAD (5.0-fold N=9) and adult controls (4.5-fold N=6).

Conclusion: Our results show that higher levels of TYRO3 and PROS1 are associated with active and multisystem LCH suggesting that this axis could be involved in the expansion of precursor and pathological LC-like cells.

Diagnosis and detection of female carriers of chronic granulomatous disease. Instituto de investigaciones en ciencias de la salud´s experience

Diana Sanabria[1], Vivian Gimenez[1], Celia Martinez de Cuellar[2], Maria Mercedes Carpinelli[1], Sara Benegas[1]

[1]*Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, San Lorenzo, Paraguay*

[2]*Instituto de Medicina Tropical - Ministerio de Salud Pública y Bienestar Social, Asuncion, Paraguay*

dianasan54@hotmail.com; viviangb@gmail.com; zhelia.martinez@yahoo.com; ihumoral@iics.una.py; bio_sar@hotmail.es

Keywords: oxidative burst, Recurrent infections, Chronic granulomatous disease (CGD), Primary immunodeficiency (PID), dihydrorhodamine test

Introduction: The chronic granulomatous disease (CGD) is a primary immunodeficiency (PID) characterized by severe and recurrent infections, due to an alteration of the neutrophils ability of producing oxygen free radicals that are used to kill certain microorganisms. In Paraguay, the Instituto de Investigaciones en Ciencias de la Salud (IICS) is the only center that has implemented and offers to the community the dihydrorhodamine (DHR) test for the diagnosis of this pathology.

Objective: Describe the experience in CGD´s diagnosis and detection of female carriers using the DHR test.

Subjects and Methodology: Two groups of children were evaluated, 54 healthy children and 112 children with clinical features compatible with CGD that were sent to the IICS for the evaluation of neutrophils oxidative capability by flow cytometry using the DHR test. The results of the test were expressed as stimulation index (SI).

Results: The average SI for the group of healthy children was 35.9±10.1 and 39.7±13.8 (p=0.07) for the group with clinical suspicion, the CGD diagnosis was confirmed in 3 of these children. In two of the cases, the SI was 1.0 and their mothers were also identified as carriers of the mutation that cause this PID, both showed 25% of neutrophils with SI=1.0 and 75% with SI=36.5. This bimodal pattern (two populations) by flow cytometry

indicates X-linked inheritance type. In one of the families, the grandmother and an aunt (from the mother side) were also identified as carriers, the last one presented 60% of the neutrophil population with an SI=1.0. In the third kid with CGD the observed SI was 7.3 and the mother did not show a flow cytometer pattern compatible with that of a carrier, exhibiting only one neutrophil population with an SI=42.3. This case could correspond to a case of autosomic recesive inheritance or a "de novo" mutation.

Conclusion: In Paraguay´s National Center of PID 8 cases of CGD were registered from 1991 to 2013, those were diagnosed with the nitroblue tetrazolium test. The implementation and application of the DHR test allowed the detection of 3 new cases in a period of less than four years of study, this fact shows an important progress in the diagnosis of this disease, and is also useful to identify female carriers.

The Th-17 and Th1 cytokines in pediatric patients with chronic infection with trypanosoma cruzi are associated with severity

Mariana De Alba Alvarado[1], Paz Maria Salazar Schettino[1], Luis Armando Jiménez Álvarez[2], Martha Bucio Torres[1], Joaquín Zúñiga[2], Edgar Zenteno[3], Margarita Cabrera Bravo[1]

[1]Facultad de Medicina, Mexico DF, Mexico
[2]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
[3]National Autonomous University of Mexico, Cuernavaca, Mexico
marianadealba@comunidad.unam.mx; pazmar@unam.mx; jimenez.alvarez.l@gmail.com; marbu@unam.mx; joazu@yahoo.com; ezenteno@unam.mx; imay@unam.mx

Keywords: Clinical Laboratory Techniques, Cardiac, Children, Cytokines and inflammation, Chagas, Trypanosoma cruzi infection, Cronic

The ability of patients to respond to T. cruzi infection depends on the balance between the immune response and the inflammatory anti-inflammatory, and also to resolve the infection. The cytokines are important because of their contribution to the mechanisms triggering inflammation and the immune response in different stages of diseases. In this study, the concentration of cytokines was determined in Mexican rural children infected with T. cruzi, and they were classified as follows: asymptomatic and two chronic phases, asymptomatic and symptomatic or severe. The goals of this study were to determine the profile of these IL-1β, IFN-γ, TNF-α, IL-2, IL-6, IL-17 and IL-RA, IL-4, IL-10, and analyze the differences between the levels of the groups. Individuals were classified according to electrocardiographic and echocardiographic clinical-cardiological studies. Group 1 included by 13 individuals in chronic asymptomatic phase, normal ECG tracing and normal echocardiogram. Group 2 consisted of 9 cases of incipient symptomatic chronic phase, with alterations in the ECG tracing of the IRBBB (block of incomplete branch of the right bundle) and echocardiography with early lesions. Group 3 included 11 patients with severe chronic phase symptomatic lesions with an IRBBB or RBBB electrocardiogram (right bundle branch block) and severe echocardiographic lesions. Individuals with chronic and severe manifestations that emerged as the majority were male (67% and 64% in symptomatic groups 2 and 3, respectively). We conclude, after multivariate analysis, that children infected with T. cruzi with severe or chronic cardiac manifestations show higher levels ($p < 0.05$) of Th17 cytokines including IL-17 and IL-6 and Th1 cytokines such as IFN-γ, that does not exist in the control healthy group;

The presence of inflammatory and fibrotic cytokines may correlate to alterations in cardiac conduction and fibrosis leading to the alteration of cardiac motility. These cytokines could be an indicator of cardiac damage in individuals infected with T.cruzi, and in the rural minor age population in Mexico.

Participation of lymphoid cells during the evolution of patients with acute myocardial infarction with ST segment elevation

Gabriela Damian-Morales[1], Ruben Lopez-Santiago[1], Nayeli Goreti Nieto-Velázquez[2]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[2]Hospital Juárez de México, Mexico City, Mexico

damianunam@hotmail.com; rlopezsantiago@hotmail.com; goretinieto@gmail.com

Keywords: Cardiovascular Diseases, Myocardial Infarction, ST elevation, Lymphoid cells, Coronary blood

Within chronic diseases, cardiovascular ones especially ischemic heart disease, is the leading cause of death worldwide. According to the INEGI, cardiovascular diseases are the first cause of mortality in the Mexican population.[1] In 2007, the European Society of Cardiology, the American College of Cardiology, the American Heart Association and the World Heart Federation; defined acute myocardial infarction (AMI) as myocardial cell death due to prolonged ischemia.[2] In this work we studied patients with type 1 AMI (spontaneous myocardial infarction, an episode related to rupture, fissure or erosion of the atherosclerotic plaque and production of a secondary thrombus in one or more coronary arteries, with necrosis of myocytes) since this is an event that occurs on daily practice, in which it is known that immediate therapy is percutaneous coronary intervention (PCI).[3] Type 1 AMI is classified into AMI with ST segment elevation (STEMI) and AMI non-ST segment elevation (NSTEMI). The ST segment in the electrocardiogram is presented as an isoelectric point, so sudden changes on it are the manifestation of an acute coronary syndrome (infarction, angina) or the rupture of arteriosclerotic plaque. Thus, the persistent elevation of ST segment in the electrocardiogram is due to a complete obstruction of the coronary flow.[4,5] Several studies have found that during STEMI the innate immune response participates in the process, initially with humoral components such as complement proteins, followed by the release of damage associated molecular patterns, reactive oxygen species, proinflammatory cytokines and hormones, among other mediators. This is followed by migration and infiltration of cells from innate immune response (neutrophils, monocytes and natural killer cells (NK)) and activation of resident cells, from the local immune response (tissue macrophages and resident lymphocytes), as well as cardiomyocytes, fibroblasts and endothelial cells, through pattern recognition receptors, which activates the NRLP3 inflammasome complex, generating cytokines

(IL-1β, IL-18 and IL-33) that alert the immune system of the damage produced. The contribution of adaptive response in AMI has been for a long time relegated to the background, behind the innate immune response. Firstly, because the infiltration of lymphocytes in the myocardium is considered an unusual event and secondly, lymphocyte activation contradicts the classical notion that adaptive immunity is not stimulated by auto-antigens.[6,7]

Therefore, this work aims to study the frequency and post-stimulation response of different lymphoid populations (CD8+ T cells, CD4+ T cells, γδ TCR, ILCs, NK, NKT, MAIT and B cells) from peripheral blood and artery coronary blood, to associate these variables with the clinical outcome of patients with STEMI. The quantification of cell populations was obtained by flow cytometry and data obtained were analysed by Kruskal Wallis statistical test.

We observed (Figure 1) that there are statistically significant changes between the percentages of different lymphoid cell populations of patients (n=17) with respect to the control group (n=6), however, this may be due to the dispersion of data obtained from the control group, which is why it is suggested to add more subjects to this group.

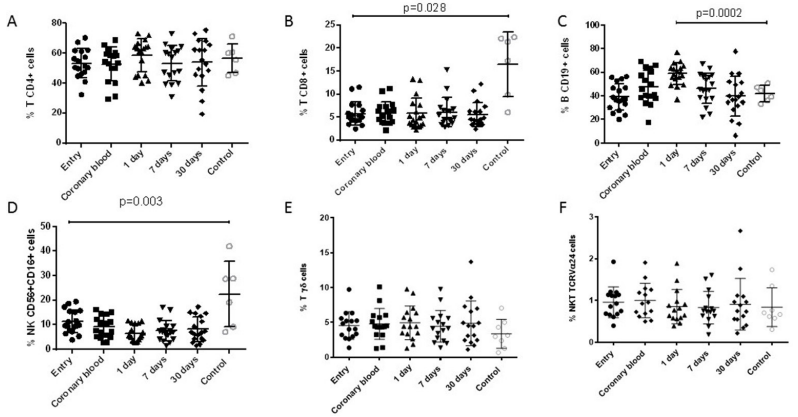


FIGURE 1: Percentage of (A) CD4+ helper T cells, (B) CD8+ cytotoxic T cells, (C) B cells, (D) NK cells, (E) γ**δ** TCR cells and (F) NKT cells, from 5 different samples during the follow-up of 17 patients with STEMI (black shapes), and the percentages obtained from 6 healthy donors – control group – (white circles). PBMCs were attained with mAb. Analysis was performed using the Kruskal Wallis statistical test. *P* values of significant differences between the groups were written.

The patient follow-up was performed upon admission and on days 1, 7 and 30 post infarction, there was no significant changes between percentages of lymphoid cells studied during the follow-up. Coronary blood was obtained during PCI, we compare the percentages of CD8+ T cells, CD4+ T cells, γδ TCR, ILCs, NK, NKT, MAIT and B cells with data obtained from peripheral blood at admission and at days 1, 7 and 30 after infarction, we found no statistically significant changes between lymphoid cell populations percentages. A previous study by Yan, et al, [8] reported differences on percentages of immune cells post infarction in a murine model, where the predominant population were CD4+ T cells, while populations with lower percentages were CD8+ T cells, γδ TCR, NK and NKT cells, 7 days after infarction. They also observed a tendency to increase each cell population at day 7 post infarction, returning to basal on day 14. However, these results, unlike ours, have been obtained from a murine model, this could partly explain the disagreement between the data found.

REFERENCES

1. Geografía, I.N.d.E.y. Principales causas de mortalidad por residencia habitual, grupos de edad y sexo del fallecido. 2014; Available from: http://www.inegi.org.mx/est/contenidos/proyectos/registros/vitales/mortalidad/tabulados/PC.asp?t=14&c=11817.
2. Thygesen, K., et al., Universal definition of myocardial infarction. Circulation, 2007. 116(22): p. 2634-53.
3. Thygesen, K., et al., Third universal definition of myocardial infarction. Glob Heart, 2012. 7(4): p. 275-95.
4. Pierard, L.A., ST elevation after myocardial infarction: what does it mean? Heart, 2007. 93(11): p. 1329-30.
5. Vaturi, M.M. and M.Y. Birnbaum, The use of the electrocardiogram to identify epicardial coronary and tissue reperfusion in acute myocardial infarction. J Thromb Thrombolysis, 2000. 10(1): p. 5-14.
6. Kawaguchi, M., et al., Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury. Circulation, 2011. 123(6): p. 594-604.
7. Chavez-Sanchez, L., et al., Innate immune system cells in atherosclerosis. Arch Med Res, 2014. 45(1): p. 1-14.
8. Yan, X., et al., Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. J Mol Cell Cardiol, 2013. 62: p. 24-35.

Approaches to endogenous intoxication evaluation in patients with facial phlegmons

Iosif A. Boev, Gleb I. Antakov, Galina I. Straube, Anatoliy P. Godovalov

Perm State Medical Academy, Perm, Russia
iosifboev@gmail.com; glebreanimator@inbox.ru; GStraube@mail.ru; AGodovalov@gmail.com

Keywords: Inflammation, leucocytes, Endogenous intoxication, Integral parameters, facial phlegmons

A steady growth of common and progressive phlegmons, as well as an increase in the number of severe complications is marked [3]. These ailments are accompanied by the development of endogenous intoxication (EI) that violates the homeostasis and induces malfunction of patient's vitally important organs and systems. The degree of EI depends on prevalence and severity of the disease.

Aim of the research – to evaluate the intensity of EI in patients with face phlegmons using a complex of integral parameters.

Materials and Methods: Retrospective analysis of 55 patient medical records with diagnosed face phlegmon (L03.2) was executed. The control included 26 healthy people. To characterize EI we used the complex of leucocyte indices [2]. Leucocyte index of intoxication (LII) is the ratio of cells' level that increases in inflammatory and purulent processes to cells, the number of which in these processes can be reduced. LII is computed according to Ya.Ya. Calf-Caliph formula:

$$LII = (4\ myel.+3\ ygn.+2\ stn.+sn.)\times(plc.+1)/(lymc.+mon.)\times(eo.+1),$$

where myel. – myelocytes, ygn. – young neutrophiles, stn. – stab neutrophils, sn. – segmented neutrophils, plc. – plasma cells, lymc. – lymphocytes, mon. – monocytes, eo. – eosinophils.

The resistance index of a body is the ratio of a number of cells to the product of the patient's age and LII. It is evident, when the index decreases below 50 points it is necessary to carry out a long detoxification therapy involving hemosorption, enterosorption, and every second patient with such a law index obtains the development of various complications.

Intoxication severity factor (ISF) is computed according to the formula:

$$ISF = neutrophils/lymphocytes.$$

For proper statistical test of the obtained data we used Student's t–test unpaired, Fisher's exact test, chi-squared test (χ^2-criterion). To assess the influence of qualitative features, an odds ratio with the 95% confidential interval was calculated. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion: The LII allows not only to express changes in DWBCC with one value, but also to evaluate the response of an organism as a whole, and also serves as a generally accepted criterion for the diagnosis of endotoxicosis in various diseases. It is well known, that LII equal to 1–3 is considered to be a norm for the majority of healthy people. The study revealed that in 26 (46.4%) patients with diagnosed face phlegmon, the LII did not exceed 3 and made in average 1.42±0.16 relative units (r.u.). All these patients were included in Group 1. In other 30 patients, who were included in the Group 2, the index value elevated 3 (6.61±0.71 r.u.; $p < 0.05$ to the Group 1). In the control group the LII made 0.60±0.04 ($p < 0.05$ to the Groups 1 and 2). The comparable groups of the patients varied in bacterial flora composition of the phlegmon secretion. Thus, among the etiologic agents in Group 1 in half of cases Staphylococcus spp. were distinguished. In this group the gram-negative microorganisms were not marked. In 19.3% cases the growth of microorganisms was not revealed. In Group 2 Streptococcus spp. was more often detected in comparison to the Group 1, and staphylococci occupied the second rank place. Gram-negative microorganisms were represented by Escherichia coli and Acinetobacter lwoffii, as well as by Neisseria. When assessing the blood clinical analysis it was revealed that in Group 2 patients there was a more pronounced left shift in DWBCC. The number of monocytes in compared groups had no statistically significant variation. In Group 2 relative lymphocytopenia was determined. It was established that EIhas a greater expression in the Group 2. For the Group 2 it is typically to have a low value of the resistance index (8.80±0.66, in control – 67.09±6.93; $p < 0.05$). It is known that the decrease of the index below 50 in the most patients provokes complications development. Apart of it, the resistance index depletion highlights the need to perform detoxification therapy. The ISF correlated with the level of severity of endogenous intoxication. Thus, in Group 1 the ISF made 3.38±0.38, and in Group 2 – 11.63±1.73 (in control – 1.68±0.10; $p < 0.05$). The conducted research established, that in 53.6% of the patients with face phlegmon the pronounced EI was developed ($p < 0.05$). The chances of development of EI at a phlegmon are 30 times higher than in the comparison group (95% CI 3.80-236.61). The relative risk of the EI development at a phlegmon is 1.94 (95% CI 1.46-2.56). Intoxication development can be stipulated both by the microbial metabolites and breakdown products of its own tissues and by

the failure of the system of mononuclear phagocytes. Thus, this study uncovers the relationship between the development of EI with the presence of streptococci and a number of gram-negative microorganisms (E. coli and other). It may be assumed that the presence of exometabolites of microbial origin in the media, the synthesis of which may vary in mixed cultures, affects the functional activity of neutrophils and other effectors of immunity. This condition is confirmed by the fact that in patients with face phlegmon there is no increase in the number of monocytes. It is certain that, it is particularly the phagocytes mononuclear system that provides the cleaning of the endogenous "wastes", the accumulation of which leads to intoxication. Beside this, the enlargement of the period from the disease onset up to the hospitalization contributes to a greater accumulation of toxic products [1].

Conclusion: In general, since the indexes of intoxication take into account all leucocytes indices of the DWBCC, it becomes easy to get holistic view of the patient's state. In addition, on the basis of the received data we may predict the course of the disease and design the treatment plan. It is known that the clinical signs of inflammation in patients may disappear early enough, however, the phenomenon of EI are preserved, what can weigh down the course of inflammatory diseases of any localization. In this respect, a timely and integral evaluation of the intensity of EI is beyond doubt.

REFERENCES

1. Godovalov AP, Bykova LP, Nikulina EA, Ozhgibesov GP, Larin AE, Larina PM. Identification of Staphylococcus aureus with a change in the microbiocenosis of the large intestine. Experimental & clinical gastroenterology. 2016; 12 (136): 36-38.
2. Ostrovsky VK, Mashchenko AV, Yangolenko DV, Makarov SV. Indicators of blood and leukocyte index of intoxication in assessing severity and determining the prognosis for inflammatory, purulent and purulent-destructive diseases. Russian Clinical Laboratory Diagnostics. 2006; 6: 50-53.
3. Shtraube GI, Boev IA, Godovalov AP, Antakov GI. Some clinical and epidemiological aspects of phlegmon maxillofacial area // Russian Dental Journal. 2017; 21 (5): 241-244.

Association study between HLA genes and climatic droplet keratopathy (CDK) in a cohort from the patagonian region of argentina

Gonzalo Montero-Martin[1], Maria Fernanda Suarez[2], Kalyan Mallempati[1], Marcelo Fernandez-Viña[1], Julio Alberto Urrets-Zavalía[3], Horacio Marcelo Serra[2]

[1]Stanford University, Stanford, United States
[2]Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[3]University Clinic Reina Fabiola, Universidad Católica de Córdoba, CORDOBA, Argentina
gmonter2@stanford.edu; mf.suarez10@gmail.com; kalyanm@stanford.edu; marcelof@stanford.edu; julioaurrets@gmail.com; hserra@fcq.unc.edu.ar

Keywords: Corneal Diseases, Environmental Exposure, Inflammation, etiology, HLA genes

Introduction: Climatic Droplet Keratopathy (CDK) is an acquired degenerative disease predominantly affecting males over 40 years old. It results in progressive corneal opacities usually affecting both eyes. CDK is multifactorial and its etiology remains unknown. Recent findings are consistent with CDK pathology being driven by environmentalfactors with oxidative stress playing an important role (e.g. contributing to lipid peroxidation) rather than climate factors (e.g. certain geographical regions of the world which share in common low humidity, constant winds and chronic exposure to high levels of ultraviolet radiation (UVR)) [1–3].We have previously found a lack of correlation between mitochondrial DNA and Y-chromosome diversity (maternal and paternal haplogroups and lineages) and the expression of CDK in populations living inArgentine Patagonia[4]. The possiblecontribution of immunogenetic factors (such asHLA class I and class II genes)to the etiopathology of CDK remainsstill understudiedas well as in many other eye diseases [5–6].

Aim: The goal of this study was to investigate the association between HLA class I (-A, -B, -C) and HLA class II (-DPA1, -DPB1, -DQA1, -DRB1, -DRB3, -DRB4, -DRB5) genes in individuals diagnosedwith grade 2 of Climatic Droplet Keratopathy (CDK) and healthy controlsfrom El Cuy Department in ArgentinePatagonia.

Methods

Samples Collection

The collection of samples consisted of both 20 healthy controls,who did not presentany pathology in the anterior segment of the eye (17 males and 3 females), and 20 grade 2 CDK cases (16 males and 4 females) from the Patagonian region of Argentina.This study was approved by the Institutional Review Board of the Catholic University of Córdoba, and the Institutional Research Ethics Committee of Health, Ministry of Health of the Province of Córdoba, Argentina (recorded in the RePIS), and carried out in accordance with the principles of the Statement of Helsinki. All subjects who were over 40 years of age provided written informed consent prior to their inclusion in the study.

HLA Class I and II typing by using a Next-Generation Sequencing (NGS) based genotyping method

All samples were genotyped for HLA-A, -B, -C,-DPA1, -DPB1,-DQA1, -DQB1, -DRB1 and -DRB3/4/5loci using the MIA FORATM NGS HLA Typing 11 Kit-96 Tests (Immucor, Inc. Norcross, GA, USA), following manufacturer's automated protocol and as it is reported in Wang et al. [7]. Briefly, sequencing reads were generated in a high-throughput platform by using this NGS-based HLA typing method (amplicon-based enrichment of HLA loci followed by massively parallel sequencing) that amplifies specifically and sequences these 11 HLA genes. Then, the bioinformatics pipeline (research version 3.1 of the MIA FORATM NGS HLA genotyping software) attributes (based on an internal reference database as well as on version 3.25 of IMGT/HLA database which contains all described HLA reference alleles sequences) these sequencing reads to specific HLA genes and calls high-resolution (atthe 4-fieldof allele resolution level) phased genotypes for each HLA gene.

Statistical Analysis

Statistical analyses were only performed at 2-fieldallele resolution level as being the first minimum level of resolution to be tested in order to see if any significant HLA allele association was possibly found in this cohort study before proceeding with further analysis at 4-field allele resolution level. Initial statistical analysis included Hardy-Weinberg testing (based on exact test of Guo and Thompson) [8], Ewens-Waterson homozygosity test of neutrality (using Slatkin's Monte-Carlo implementation of the exact test) [9][10], determination of allele frequencies and all pairwise linkage disequilibrium (LD) estimatesusing the softwareanalysisPypop (Python for Population genomics) v.0.7.0[11]. Regarding statistical association analyses: comparison of allelefrequen-

ciesin 2x2 contingency tablesand the evaluation of their significance(significance of the differences was evaluated using two-tailed Fisher's exact test [12], and P-values less than 0.05 were considered significant), as well as calculation of odds ratios (OR), relative risk (RR) and respective confidence intervals (with a 95% confidence interval (95%CI)) were calculated (according to Altman[13]) usingEpi InfoTM (version 7.0).

Results: The observed and expected frequencies between genotypes for all HLA loci showed no deviation from Hardy-Weinberg equilibrium in cases or controls, with P-values greater than 0.05. The frequencies of all HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, -DRB1, -DRB3/4/5 alleles were calculated and no significant differences were found between the two groups studied, except for the HLA-B locus, which showed a statistically significant difference for the HLA-B*51:01allele (2.6% CDK cases vs. 21.1% Healthy controls, P = 0.0284, OR = 0.101, and 95%CI = 0.012–0.856) and also for the HLA-DRB3 locus, which in this case showed a statistically significant difference for the HLA-DRB3*01:01allele (26.5% CDK cases vs. 3.8% Healthy controls, P = 0.0329, OR = 9.000, and 95%CI = 1.060–76.427).

Discussion: In the cohort studied here, our preliminary performed analyses suggest influence of certain HLA genes on resistance and susceptibility to Climatic Droplet Keratopathy (CDK). In which we have found associations for the allele HLA-B*51:01 (protection against developing of CDK) and for the allele HLA-DRB3*01:01 (susceptibility for developing of CDK).Curiously/Peculiarly, HLA-B*51:01 is the major suballele associated with Behçet's disease (BD) in all the populations studied [14]. Regarding, HLA-DRB3*01:01, Smikle, M.F.,et al have shown a very strong association between this allele and Graves' disease in Jamaican [15].At the same time, this interpretation of our results in this study may need to be taken carefully and in the context of the reduced sample size used for this study. Nevertheless, this present study has allowed us to report for the first time the diversity of all HLA class I and II genes in high-resolution and phased genotypes of this cohort (of both CDK patients and healthy controls) from this Patagonian region of Argentina. At the same time, the HLA typing results obtained from this study should be a good complement for future studies which can continue undercover the underlying mechanisms of this corneal disease.

Conclusions: In the present study, our initial performed analyses suggest a possible influence of certain HLA genes (HLA-B*51:01 protective allele and HLA-DRB3*01:01 risk allele) on resistance and susceptibility, respectively to CDK in this studied cohort from Argentine Patagonia. Nevertheless, further studies would need to be conducted for a more complete elucidation of ourdata obtained in this study.

REFERENCES

[1] Serra, H.M., Holopainen, J.M., Beuerman, R., Kaarniranta, K., Suárez, M.F. and Urrets-Zavalía, J.A. (2015). Climatic droplet keratopathy: an old disease in new clothes. ActaOphthalmologica, 93(6), pp.496-504.

[2] Suárez, M.F., Correa, L., Crim, N., Espósito, E., Monti, R., Urrets-Zavalía, J.A. and Serra, H.M.(2015). Climatic droplet keratopathy in Argentina: involvement of environmental agents in its genesis which would open the prospect for new therapeutic interventions. BioMed Research International, 2015.

[3] Suarez, M.F., Piqueras, M.C., Correa, L., Esposito, E., Barros, M.F., Bhattacharya, S.K., Urrets-Zavalia, J.A. and Serra, H.M. (2017). Phospholipidomic Studies in Human Cornea From Climatic Droplet Keratopathy. Journal of Cellular Biochemistry.

[4] Schurr, T.G., Dulik, M.C., Cafaro, T.A., Suarez, M.F., Urrets-Zavalia, J.A. and Serra, H.M. (2013). Genetic background and climatic droplet keratopathy incidence in a Mapuche population from Argentina. PloS One, 8(9), p.e74593.

[5] Levinson, R.D. (2005). Immunogenetics of ocular inflammatory disease. In Uveitis and Immunological Disorders (pp. 91-107). Springer, Berlin, Heidelberg.

[6] Goverdhan, S.V.,Lotery, A.J. and Howell, W.M. (2005). HLA and eye disease: a synopsis. International Journal of Immunogenetics, 32(6), pp.333-342.

[7] Wang, C., Krishnakumar, S., Wilhelmy, J., Babrzadeh, F., Stepanyan, L., Su, L.F., Levinson, D., Fernandez-Viña, M.A., Davis, R.W., Davis, M.M. and Mindrinos, M. (2012). High-throughput, high-fidelity HLA genotyping with deep sequencing. Proceedings of the National Academy of Sciences, 109(22), pp.8676-8681.

[8] Guo, S. W., and Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics, 361-372.

[9] Slatkin, M. (1994). An exact test for neutrality based on the Ewens sampling distribution. Genetics Research, 64(1), 71-74.

[10] Slatkin, M. (1996). A correction to the exact test based on the Ewens sampling distribution. Genetics Research, 68(3), 259-260.

[11] Lancaster, A.K., Single, R.M., Solberg, O.D., Nelson, M.P. and Thomson, G. (2007). PyPop update–a software pipeline for large-scale multilocus population genomics. HLA, 69(s1), pp.192-197.

[12] Fisher, R.A., 1922. On the interpretation of χ 2 from contingency tables, and the calculation of P. Journal of the Royal Statistical Society, 85(1), pp.87-94.

[13] Altman DG (1991) Practical statistics for medical research. London: Chapman and Hall.

[14] Kaya, T.I. (2012). Genetics of Behçet's disease. Pathology Research International, 2012.

[15] Smikie MF, Pascoe RW, Barton E, Morgan O, Christian N, Dowe G, Roye-Green K, Bailey V, James O. HLA-DRB3*0101 is associated with Graves' disease in Jamaicans. Clin Endocrinol (Oxf). 2001 Dec;55(6):805-8.

Dengue virus replication is inhibited in chikungunya and dengue co-infected mexican infants

Verónica López-Perrusquía[1], Mussaret Bano-Zaidi[2,3], Julio García-Cordero[1], Josselin Carolina Corzo-Gómez[1], María Elena González y Almeida[2], Nicolás Villegas-Sepúlveda[1], Leopoldo Flores-Romo[1], Leticia Cedillo-Barrón[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
[2]Hospital General O'Horan, Merida, Mexico
[3]Michigan State University, East Lansing, United States
verotoxi@hotmail.com; mussaret_zaidi@yahoo.com; leebeydengue@yahoo.com.mx; jcorzo@cinvestav.mx; maria.gonzalez@ssy.gob.mx; nvillega@cinvestav.mx; leflores@cinvestav.mx; lcedillo@cinvestav.mx

Keywords: Chikungunya virus, Coinfection, Dengue Virus, infants, mexican, seroconversion

Background: Dengue and Chikungunya are global public health threats. Dengue may cause dengue shock syndrome or hemorrhage that can lead to death, while Chikungunya can cause intense joint pain. Dengue (DENV) and Chikungunya (CHIKV) are RNA viruses transmitted to humans through the bites of infective female Aedes mosquito. Vector competence studies reveal that Aedes mosquito is capable of transmitting both DENV and CHIKV simultaneously1, and as a result, co-infecting humans. Co-infections can either facilitate or hinder viral replication and transmission and might also affect the severity of infection and the immune response of the host. Although DENV-CHIKV co-infection cases have been documented in many countries (including Mexico), little is known about DENV-CHIKV interactions and its possible association with severe clinical disease. Many reports have suggested a more severe clinical outcome for DENV-CHIKV co-infected patients when compared to mono-infected ones2; but in other studies there is not an association between co-infection and severe disease3. Notably, all these studies involved the East/Central/South African CHIKV genotype. In 2015, Yucatan State Health Department reported 1669 confirmed cases of Chikungunya fever. During the same year, DENV serotypes 1, 2 and 4 widely co-circulated throughout the state. In that year, during August to October 2015, the Hospital General O'Horan in Merida, Yucatan, witnessed a sudden and massive influx of patients with acute febrile illness and severe joint pain; a significant proportion of young infants required hospitalization. We wanted to determine whether these patients presented DENV-CHIKV co-infection and if this was associated with greater severity of disease or altered immune response.

Methods: We analyzed blood samples for 24 patients (aged 2 months to 82 years) admitted to the hospital during the height of the Chikungunya outbreak. All patients presented fever greater than 38°C and at least two or more of the following symptoms: rash, arthralgia, myalgia and headache. DENV and CHIKV were identified by RT-PCR; IgM and IgG antibodies were detected by ELISA. CHIKV PCR products were cloned, sequenced and subjected to BLAST analysis.

Results: Only 14 patients had positive PCR results: 3 for DENV, 5 for CHIKV and 6 for DENV and CHIKV. After 4–6 weeks of infection, we obtained convalescent serum samples from 21 of the 24 patients. Among the 3 DENV PCR positive patients, 2 presented DENV and CHIKV specific IgM in their acute samples, and DENV and CHIKV specific IgG in the convalescent ones. Only 1 CHIKV PCR positive patient presented seroconversion to both viruses. The combined PCR and serology results yielded 9 DENV-CHIKV co-infections, 2 DENV infections and 4 CHIKV infections. 8 children were DENV and CHIKV PCR positive, of whom five, who were infants less than 2 years old, only presented seroconversion of CHIKV-specific IgG antibodies. The other 3 children (5–8 years old) and a young adult seroconverted to both viruses. No clinical differences were observed between mono and co-infected patients. Of the 6 patients with a positive DENV and CHIKV amplicon, two RT-PCR products were cloned. We chose three colonies from each patient for sequencing and phylogenetic analysis (CHIKV E2 region was used). The nucleotide sequences of our two isolates possessed 100% identity among each other, and one isolate (accession number MF407264) was shown to have 100% homology with the viral sequences previously reported for an isolate from Chiapas, Mexico (accession number KP851709.1). Analysis of CHIKV PCR products by BLAST showed that our isolates belonged to the Asian type.

Discussion: The IgG seroconversion of co-infected young infants to CHIKV alone is an interesting finding. The lack of conversion in these infants could be due to suppression of DENV replication by CHIKV, since the latter replicates and disseminates faster. In consequence, CHIKV may trigger and control the innate immune response (mainly type I interferons) before DENV can induce it. By activating the antiviral response against CHIKV in the infected and neighboring cells DENV infection could be altered by the activation of this response, primarily the presence of interferon β and α. Interestingly, our CHIKV isolates belonged to the Asian genotype which could also explain the observable outcome. Another explanation could be the presence of circulating maternal anti-DENV but not anti-CHIKV. These maternally derived antibodies appear to play a strong protective role against dengue infection in young infants. Also, the intrinsic differences in the immune response of young infants compared to older children and adults could play an important role in this event. It has been observed

that young infants are poor IgG responders towards protein and polysaccharide antigens; hence it is plausible that our young infants were not capable of detecting DENV antigens at low levels. Finally, we could not see an association between co-infection and severe disease.

Conclusions: Our results are the first detailed description of DENV-CHIKV co-infected young Mexican infants and provide evidence that the viral dynamics and immune response in this age group differs from that of older children and adults. The lack of DENV seroconversion in the co-infected infants might be explained by the presence of maternal antibodies, viral suppression of DENV by the Asian lineage CHIKV or the immature response in young infants.

Common variable immunodeficiency. About a case report

Maité Pérez Cutiño

Universidad de Ciencias Médicas de la Habana, Havana, Cuba
mperezc@infomed.sld.cu

Keywords: Common Variable Immunodeficiency, genetic disorders, Clinical Immunology, Primary immunodeficiency, Recurrent infections

Introduction: Common variable immunodeficiency (IDVC) is a relatively frequent primary immunodeficiency due to antibody deficiency. It is considered a genetic disorder due to the presence of mutations in genes that code for a particular component of the adaptive immune system. It is characterized by a decrease in serum levels of IgG, IgA and sometimes IgM. The clinical picture is highly variable and four clinical phenotypes are described.

Case presentation: A 16-year-old patient, who presented from the first year of life had high and low respiratory infections, complicated and uncomplicated. There were more than 10 episodes in a year, mainly of bacterial etiology, with multiple admissions and broad-spectrum antimicrobial treatments. Personal and family history of asthma, atopic dermatitis and recurrent eczemas were reported. He also presented gastrointestinal disorders of different causes and milk intolerance. On one occasion, he was admitted for an adenian syndrome, which was interpreted as an infectious mononucleosis. Immunological studies were performed due to a complicated pneumonia finding a decrease in serum IgG levels and a decrease in the CD19 positive CD19 lymphocyte population was confirmed by flow cytometry. He was treated with intravenous gammaglobulins with good response.

Conclusions: In the presence of bacterial infections, mainly of bacterial origin, it is important to think about this disease, since diagnosis and timely treatment improve the patient's quality of life.

IgG antibody Index against Epstein Barr in Multiple Sclerosis patients

Jose Alejandro Rodríguez-Pérez, Vanessa Pérez-del-Vallín, Alejandro Mirabal-Viel, Cristobal González-Losada, Alexander Ariel Padron-Gonzalez, William Castillo-González, Alberto Juan Dorta Contreras

Universidad de Ciencias Médicas de la Habana, Havana, Cuba
frcala@infomed.sld.cu; vanessavallin@nauta.cu; alecuba95@nauta.cu; chriss.losada@gmail.com; paxander@infomed.sld.cu; williamcg@infomed.sld.cu; adorta@infomed.sld.cu

Keywords: Multiple Sclerosis, IgG, Epstein Barr virus, antibody index, reibergrama

Abstract

Introduction: Cause of Multiple Sclerosis is not well known yet. There are theories that point out a viral origen that may initiate the autoimmune response. There are a lot of biological agents that could be causative. Among them is Epstein Barr Virus but there are controversies about this topic. With the evaluation of the specific antibody index developed by Reiber, perhaps it will be possible to find some evidences that sign Epstein Barr virus like the cause of the disease.

Objective: The aim of this paper is to evaluate the antibody index anti-Epstein Barr virus in 27 patients with multiple sclerosis and 19 control cases.

Results: There were significant differences between Q specific (Specific antibodies in Cerebrospinal fluid / Specific antibodies in serum) between the sclerosis multiple group and the control group ($p < 0.0001$). The antibody index(Q specific/Q IgG or Q lim) between both groups was not significant differences ($p=0.0519$).

Conclusions: Anti- Epstein Barr Q specific in Multiple Sclerosis could contribute to clarify the etiology of this disease but it is necessary to increase the total of patients involved in the sample in order to confirm the causative of the disease.

Introduction: Multiple Sclerosis (MS) is a chronic demyelinating disease that produces disabilities and affects mainly young adults. It is a complex disorder and it involves inflammation, neurodegeneration and axonal restoration.

The biological agent hypothesis and the transmission possibility of MS are generating several controversies in the medical literature. There are a large list of candidates to be the causative of the disease such as rabies, herpes virus, influenza virus type 1, measles, cytomegalovirus, coronavirus and retrovirus. Several factors support this hypothesis such as a premature exposition during infancy, neurotropism and the quick reactivation and proliferation of infected cell types.

Epstein Barr(EB) was recently described that has as an important role in the etiology of MS as an activator of the disease but the epidemiological studies are not conclusive because of previous infections in people without MS1

The auxiliary diagnosis of multiple sclerosis is using the so-called measles-rubella-zoster virus (MRZ) reaction, which is the presence of elevated antibodies against measles, rubella and herpes zoster based on a polyclonal and polyspecific activation.2

The objective of this work is to verify if there is a relationship between the specific response to Epstein-Barr given by the specific IgG antibody index and multiple sclerosis.

Materials and Methods: 27 patients diagnosed with MS and 19 control patients without neurological disorders were studied. Serum and cerebrospinal fluid samples were taken simultaneously and stored refrigerated at minus 80 degrees until use. The samples comes from LABCEL collection library Serum albumin and IgG were quantified by simple radial immunodiffusion plates Partigen (Siemens, Marburg) and in cerebrospinal fluid (CSF) were determined using LC Partigen plates of the same firm.

IgG against EB were determined by (ELISA Enzygnost anti EBV IgG, Siemens, Marburg). To know if the patients had intrathecal IgG synthesis, the Reibergrams were employed. The antibody index was calculated from the formula described3

MedCalc 16.0 statistical software package were used for statistical purposes.

This research follows ethical standards and was approved by the Research Ethics Committee Miguel Enríquez School of Medical Sciences.

Result: Figure 1 shows the Reibergram where the white squares are the patients with Multiple Sclerosis and the black triangles the control cases. The cases of MS present intrathecal IgG synthesis.

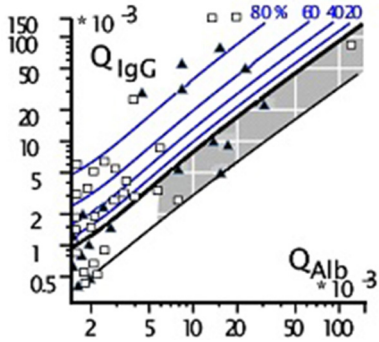


FIGURE 1: CSF/serum quotient diagrams for IgG (Reibergram). The upper hyperbolic curves (thick lines) represent the discrimination lines between brain-derived and blood-derived immunoglobulin fractions. Values above these upper discrimination lines represent intrathecal IgG synthesis. The dashed lines indicate the extent of intrathecal synthesis as intrathecal fractions (IgGIF) with 20, 40, 60, and 80% of the measured total immunoglobulin concentration in CSF, with reference to the discrimination line as 0% intrathecal synthesis. The plotted white square represent EM patients and black triangles mean the controls.

Table 1: Q Values and Antibody Index anti E B

	Q-EB-COM	Q-EB-EM	Q-lgG-COM	Q-lgG-EM	IA-COM	IA-EM
N	0.1720	0.9146	3.5000	2.4000	0.0800	0.8300
DS	0.4345	0.2472	21.7391	134.6383	0.3839	2.6300
X	0.4457	0.8300	15.6316	40.8589	0.2849	0.9146
P*	accept Normality (P=0.124)	accept Normality (P=0.452)	reject Normality (P=l.041)	reject Normality (P<0.001)	accept Normality (P=0.147)	reject Normality (P=0.002)

*Kolgomorov Smirnov test

Table 1 shows the variables including arithmetic mean and standard deviation for Q specific EB, Q IgG and EB Antibody Index, in control cases and multiple sclerosis patients.

Mean comparison was performed between variables and it can be observed in Table 2.

Antibody Index in MS patients had not significant difference with the Antibody Index in controls. However there are quite a good significant difference when both quotients of IgG specific had been compared (Table 2).

Table 2: Comparison anti-Epstein Barr Antibody Index (AI) and Q-EB specific lgG

	AI MS*	AI C*	Qs MS**	Qs C**
Mean	1.5	0.28	0.91	0.44
Standard deviation	2.63	0.38	0.24	0.43
n	27	19	27	19

*t=-1.99 p=0.0519 **t=-4.59 p<0.0001

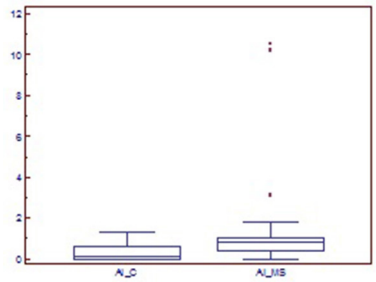


FIGURE 2: Antibody Index Box-and-Wisker graphs. AI_Con Antibody Index Controls; AI_MS Antibody Index MS patients.

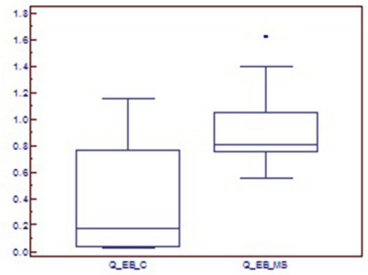


FIGURE 3: Q values Box-and-Wisker graphs. Q_EB_C: Q specific EB in controls; Q_EB-MS: Q specific in MS patients.

The mean results of the levels of the antibody index can be seen in Figure 2. There is a non-significant difference between the control and the MS cases.

In Figure 3 show the box-and-wisker graph of the two quotients studied. Notice also from Table 2 that there were significant differences between the two groups.

Discussion: There is a controversy not yet elucidated regarding the association of Epstein Barr and multiple sclerosis. The results obtained in a preliminary way did not show significant differences in terms of the antibody index mean levels. So the controversy persists and it is not possible to verify the hypothesis. This index could have a diagnostic value if the anti-EB antibody indexes have been higher than 1.5, which could not be verified in all patients. 3,4

The association between EB infection with MS could have more chance to be significant if the existence of further risk factors like age.5 Having mononucleosis clinical manifestations also implies slightly more risk of MS than those asymptomatic forms.

Other virus were reported to have relation with MS like measles, varicela zoster virus, herpes simplex virus and other ones 6, Genetics risks, low Vitamin D levels, smoking, among other ones could be associated also with the disease..7

EB is a wide spread virus that affects 95% of the world population and only a little proportion develops the disease. It has been suggested that EB could be a risk factor not the causative of the disease.8

There are no evidences that EB is present in brain tissue of EM patients or the existence of cross reactivity due to molecular mimetism of the anti-EB antibodies against antigens proper from the central nervous system that could initiate a local autoimmune response.9

These results may be due to the limited size of the sample. The relationship between specific IgM anti-EB in these patients has not been studied, and perhaps it could help to verify this association. However, differences could be seen, although were not significant, regarding the mean levels of the antibody index.

REFERENCES

1. Puertas S, del Carmen O. Presencia del virus de Epstein-Barr en la esclerosis múltiple.[Tesis Doctoral]. Granada España: Editorial de la Universidad de Granada; 2010.
2. Robinson-Agramonte M, Reiber H, Cabrera-Gomez JA, Galvizu R. Intrathecal polyspecific immune response to neurotropic viruses in multiple sclerosis: a comparative report from Cuban patients. Acta Neurol Scand. 2007 May; 115(5):312-8.
3. Dorta-Contreras A, Bu-Coifiu-Fanego R, Magraner Tarrau ME, et al. Neuroinmunología Clínica. Habana: Academia, 2009. pp:1-223 ISBN 959-270-147-2
4. Lassmann, H. et al. Progressive multiple sclerosis: pathology and pathogenesis. Nat. Rev. Neurol. 2012; 8, 647–656.
5. Handel AE, Giovannoni G, Ebers GC, Ramagopalan SV. Environmental factors and their timing in adult-onset multiple sclerosis. Nat. Rev. Neurol. 2010; 6:156–166.
6. Fernánndez AM, Fernández V, Guerrero M, Fernández O. Papel de las infecciones en la esclerosis múltiple. Rev Esp Escler Mul.2010; 2(13):10-6.
7. Sundqvist E, Sundström P, Lindén M, Hedström AK, Aloisi F, Hillert J, et al. Epstein-Barr virus and multiple sclerosis: interaction with HLA. Genes and immunity.2012; 13(1):14-20.

8. Kelly Claire, O'ReillyEilisJ,MungerKassandra L, Susan Finerty, Andrew J Morgan, Alberto Ascherio. Epstein–Barr virus neutralizing antibody levels and risk of multiple sclerosis. MultSclerAugust 2012; 18(8): 1185-1187.
9. Calabrese Massimiliano , Magliozzi Roberta , Ciccarelli Olga , G. Geurts Jeroen J ,Reynolds Richard Martin Roland . Exploring the origins of grey matter damage in multiple sclerosis. NatRevNeurosc 2015; 16 :147-158.

Global Clinical Trials Connect 2018

Ebi Ebi John

Corvus Global Events, London, United Kingdom
ebi.corvus@gmail.com

Keywords: clinical trials, clinical research, Site selection, Artificial intelligence technology, Clinical technology, patient recruitment, clinical trial audit, patient centricity, Big data and IoT Clinical Trials, Adaptive Trial Model

The Global Clinical Trials Connect 2018 conference focuses on introducing pioneer technology, developing better patient engagement and collaborating strategies in clinical trials. With the right commitment and attention to detail, contract research organizations (CROs) and pharmaceutical companies can drive collaboration with greater efficiency.

The global pharmaceutical industry has seen a downturn in recent years because of the challenges and cost associated with pharmaceutical development, procrastination in drug development, etc. Clinical trials field is also faced with such challenges. Cases of failures, cost and delay are high. It is high time that we look into innovative strategies, new technologies, effective and quality collaborations to address these issues, which can cater to the needs of the patient and the industry. Due to complex clinical trials and bygone data standardization methods, we need algorithms and lucrative strategies that will enhance the clinical trials outcomes. There are vast data collected across clinical trial process, the standardization of these data will turn into an opportunity for companies to trap the information and raise clinical trial design, patient recruitment, monitoring insights and augment decision-making.

One response to this can be CROs and pharmaceutical companies can together work on diverse and varied clinical trial activities, also in near future we require joint effort for fulfilling outsourcing requirements and providing effective clinical trials.

This conference will bring together the industry experts and leaders across pharmaceutical, biotechnological and CRO's for brainstorming on Case studies on innovation, collaboration and existing clinical data to headway the Clinical Trials process. Ethical considerations are given in this context and for this RBM are beginning to extend its concepts in Quality Risk Management; clinical trial predictive models are accumulated by enterprises, methodologies in trial design and many more.

It gives us a great pleasure to welcoming you to the Global Clinical Trials Connect 2018.

Serological markers of viral and autoimmune hepatopathy in coelic patients

Deyanira La Rosa Hernández

Sociedad Cubana de Inmunlogía (SCI), Habana, Cuba
deyani@infomed.sld.cu

Keywords: Viral Hepatitis, aminotransferases, Celiac desease, Transglutaminase antibodies, autoinmune hepatitis

Introduction: Celiac disease may also associated to severe forms of liver illness and/or coexist with other chronic liver disorders.

Objetive: The aim of this study was to determine the prevalence of liver disorders in adult celiac patients.

Materials and Methods: Across-sectional study was conducted at Cuban National Institute of Gastroenterology, from March 2016 and March 2017. Forty-three patients with celiac disease were studied. Serum aminotransferases as well as as autoantibodies related to autoinmune and viral hepatitis were evaluated.

Results: Of the 43 adult celiac patientes, 74.4% were women. Median age of presentation was 51 years. Hypophosphatasemia was found in 25 (58.1%) while 6 (14 %) showed increased levels of aminotransferases.18 patients (41,8%) were positivity for antinuclear autoantibodies, 7(16,3%) of whorm presented antismooth muscle antibody (ASMA) and 3 (6,9%) antimitocondrial antibody (AMA). Hipertransaminasemia had a significant association to transglutaminase antibodies ($P < 0.01$).

Conclusions: Celiac desease is often accompanied by autoinmune hepatitis.

Characterization of the immunoallergenic profile of the low molecular weight fraction of the allergenic extract of the mite Blomia tropicalis

Maytee Mateo Morejón[1], Jesus Alberto Garcia Lerena[2], Maria del Carmen Luzardo[2], Alexis Labrada[1]

[1]Centro Nacional de Biopreparados, Havana, Cuba
[2]University of Havana, Havana, Cuba
mayteemm@biocen.cu; alberto@fbio.uh.cu; mcluzardo@fbio.uh.cu; labrada@biocen.cu

Keywords: sensitization, Blomia tropicalis, Allergenic extract, Blo t 2., immunoallergenic profile

Introduction: Blomia tropicalis (Bt) is a major causative agent of respiratory allergic diseases. In Cuba, the percentages of sensitization to Bt range from 55 to 75%. The objective of this study was to characterize the IgE binding profile of the low molecular weight fraction of the Bt allergenic extract in Cuban patients.

Methods: The low molecular weight fraction (LMWF) of Bt was obtained by precipitation with 60% ammonium sulfate from a mite whole culture extract of this species and fractionated on a Superdex 75 HR 10/30 column (PS-75). 142 allergic patients were selected showing asthma symptoms, associated to House Dust exposure, and subjected to skin prick testing (SPT) with a Bt standardized allergen extract (VALERGEN, Cuba). The IgE reactivity of the fraction was assessed by ELISA and Western blot, using sera from SPT positive patients.

Results: The LMW fraction showed high content (73%) of the 14–15 kDa bands, tentatively identified as Blo t 2 isoforms. 113 out of 142 patients were positive by SPT to B. tropicalis extract. 64% of sera from SPT positive patients showed IgE binding activity to the 14–15Kda by Western blot assay, tentatively corresponding to isoforms of the Blo t2 allergen. Moreover, 82% of SPT positive patients showed also IgE binding activity by ELISA to the LMWF, with absorbance values higher than the cut-off (0.175) and a mean value of 0.381 ± 0.062 (DO 405 nm). Taking together Western blotting and ELISA, 55% of SPT positive patients showed coincident positive results by both methods. Significant correlation values (Spearman) were observed between the size of the wheal in the SPT and the absorbance values in ELISA ($r=0.391$, $p<0.0001$).

Conclusions: The low molecular weight fraction of Blomia tropicalis is recognized by the IgE of most allergic patients sensitized to this mite, being the predominant components at 14-a5 kDa.

Evaluation of the use of biomodulina T in the treatment of patients with thymus hypoplasia in cuba

Mary Carmen Reyes Zamora[1,2,3], Regla Jorrín Caraballo[1], Katia Katia Rodríguez Gutiérrez4[4], Maytee Mateo Morejón[3]

[1]Rosa Elena Simeón Polyclinic, Mayabeque, Cuba
[2]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[3]Centro Nacional de Biopreparados, Havana, Cuba
[4]Angel Arturo Aballí Hospital, Havana, Cuba
mcarmen.reyes@biocen.cu; reglajc@infomed.sld.cu; katiarg@infomed.sld.cu; mayteemm@biocen.cu

Keywords: immunodeficiency, immunomodulator, biomodulina T, Thymus Hypoplasia, Thymus ultrasound

Introduction: Although Thymus Hypoplasia is not a disorder of high incidence / prevalence, it constitutes a condition with a high social impact, particularly on children suffering the disease and their relatives, as well as, on the health system. Currently, there are is a lack of evidence-based treatments for Thymus Hypoplasia. Biomodulina-T (BioCen, Cuba) is a thymus extract registered as an injection with indications as immunomodulator for the treatment of recurrent Respiratory Acute infections in elderly patients. For some years, Biomodulina T has been used in Cuba for the treatment of Thymus Hypoplasia as an off-label use. The objective of this study was to assess the efficacy and safety of Biomodulina T in children under 5 years with Thymus Hypoplasia.

Materials and Methods: Sixty one children with diagnostic of Thymus Hypoplasia by ultrasound and clinical manifestations of immunodeficiency were attended in "Rosa Elena Simeón" Polyclinic, in Bejucal, Mayabeque, Cuba, as a primary care level setting. All of them received intramuscular Biomodulina T for 12 weeks, at a rate of one, two or three vials per week (3 mg/vial), depending on the severity of the Hypoplasia and the clinical manifestations of the patients. Ultrasound examination was used to assess the thymus area. Other complementary immune tests were performed at least 3 months after completing the treatment.

Results: Following he treatment, a statistically significant growth of the Thymus area was evidenced ($p<0.05$). Thymus area increased from 698.8 ± 116.1 mm2 before treatment to 948.7 ± 156.9 mm2 after the same. Only in 12 patients was necessary to repeat

the treatment cycle. Erythema at the injection site was reported in three patients as a side effect, which resolved spontaneously and the treatment could be continued.

Conclusions: Biomodulina T is safe and effective treatment of Thymus Hypoplasia in children, although it is recommended to perform a controlled clinical trial to increase the level of evidence and standardize the treatment on these patients.

Low percentages of regulatory T cells in Common Variable immunodeficiency (CVID) patients with autoimmune diseases; and association with increased numbers of CD4+CD45RO+ T and CD21low B cells

Laura Berrón-Ruiz[1], Gabriela López-Herrera[1], Nora Segura-Méndez[2], Patricia O'Farril-Romanillos[2], Ma Enriqueta Nuñez-Nuñez[3], M Carmen Zarate-Hernández[4], Noemi Gómez-Hernández[5], Dolores Mogica-Martínez[6], Marco A Yamazaki-Nakashimada[1], Tamara Staines-Boone[7], Leopoldo Santos-Argumedo[8]

[1]Instituto Nacional de Pediatria, Mexico City, Mexico
[2]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
[3]Hospital Civil de Guadalajara, Guadalajara, Mexico
[4]Hospital Universitario Monterrey, Monterrey, N.L., Mexico
[5]Hospital de Occidente CMN, Guadalajara, Mexico
[6]Centro Médico Nacional La Raza, Mexico City, Mexico
[7]Centro Médico Noreste CMN, Monterrey, Mexico
[8]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
lberronruiz@yahoo.com.mx; lohegabyqbp@gmail.com; norasegura@yahoo.com.mx; patyfritzenwalden@hotmail.com; nenezen@hotmail.com; carmenzarateh@yahoo.com.mx; ngh_mimi@hotmail.com; doloresmogica@hotmail.com; yzki71@yahoo.com.mx; tamarastaines@gmail.com.mx; lesantos@cinvestav.mx

Keywords: regulatory T cells, CVID, memory B cells, memory T CD4+ cells, CD21low B cells

Introduction: Common Variable Immunodeficiency (CVID) is a heterogeneous group of primary antibody deficiencies, which is defined by marked reduction of serum IgG, IgA and/or IgM levels and recurrent bacterial infections. Recently, reports have been published indicating reduced numbers of regulatory T cells (Tregs) in CVID patients and its correlation with chronic inflammation, splenomegaly and autoimmune manifestation (1–3). Treg cells have an important role in controlling immune responses, maintaining peripheral self-tolerance; they are characterized by surface expression of CD4 and CD25 in combination with intracellular expression of the forkhead family transcription factor (FOXP3). In CVID, autoimmune diseases are present in 10–30% of patients; of these, autoimmune cytopenias are the highly common (4). More recently, it was found that half the patients with CVID having only infections had normal T-B phenotype whereas patients with abnormal T-B phenotype were significantly more likely to suffer autoimmune and lymphoproliferative diseases(5).

Objective: We analyzed whether there is an association between Treg cells and memory subpopulation of T and B lymphocytes, as well as autoimmune manifestations in 36 patients with CVID (25 women and 11 men, mean age 23 years).

Methods: To assess the different lymphocyte population and subpopulations, we performed flow cytometric analysis in controls and CVID patients prior to IVIG infusion. Lymphocyte populations were enumerated in whole-blood samples and stained with the following mixtures of monoclonal antibodies (mAb): anti CD45-FITC/anti CD14-PE, anti CD3-FITC/anti CD19-PE/anti CD45-PerCP, anti CD4-FITC/anti CD8-PE/ anti CD3-PerCP, anti CD3-FITC/anti CD19-PE/anti CD45-PerCP, anti CD3-FITC/ anti CD16+16-PE/anti CD45-PerCP; for naïve (CD45RA+) and memory (CD45RO+) T cells, the following antibodies were used anti CD45-RO-FITC/anti CD45-RO-PE/ anti CD3-PerCP/anti CD4-APC, and, γ1-FITC/ γ2-PE/ antiCD45-PerCP were used for isotype control. To identify B cell populations, peripheral mononuclear cells (PMBCs) were isolated using density gradient centrifugation with Histopaque®-1077. Isolated PMBCs were then stained with a mixture of anti CD19-APC/ anti IgD-FITC/ antiCD27-PE, antiCD19-FITC/ antiCD21-PE/ anti38-APC, antiCD19-FITC/ antiCD24-PE/ anti38-APC. The Freiburg classification, groups was based on staining of PMBCs with following antibodies, as published in (6, 7): group Ia represent patients containing an isotype-switched memory B cell fraction (CD19+/CD27+/IgD-) of below 0.4% of the total lymphocytes, along with a proportion of CD21low B cells equal to or exceeding 20% of total B cells; group Ib included patients with a low percentage of switched memory B cells as detailed above, but with normal proportion of CD21low B cells; whereas group II included patients with normal B cells subsets. Treg cells were detected by staining PMBCS with anti-human FOXP3-APC, CD25-PE, CTLA-4-PECy5 and CD4-FITC; the frequency of Tregs FOXP3+/CD25+ cells was calculated from CD4 T cells and the frequency of CTLA-4+ cells was calculated from the total FOXP3+/ CD25+ cells. An additional analysis included cell surface staining using anti-CD25-PE, antiCD4-PerCP and anti-CD127-APC; using blood lysis technique, and were defined as being CD25+/CD127low, expressed as a percentage of CD4 cells. Statistical analyses were performed using a Mann-Whitney test and Spearman's coefficient (R2) was used to determine the significance of the correlation between two variables. The results are expressed as the mean, and p-values <0.05 were considered to be significant.

Results: The demographic details of the subjects included in this study are show in Table 1. Fourteen patients were coursing with autoimmune diseases (39%); eleven with autoimmune thrombocytopenia (ITP) (31%); two with vitiligo (6%); one with systemic lupus erythematosus (LES) (3%) and one with multiple sclerosis (MS) (3%). Patients with CVID and autoimmunity had less Treg CD4+CD25 (high) CD127 (low),

Table 1: Clinical characteristics of 36 CVID patients

	Total patients (n = 36)	Patients with autoimmune cytopenias (n= 14)	Patients without autoimmune cytopenias (n= 22)
Sex (female/male)	25/11	8/6.	17/5.
Mean of age	24	21	25
Pneumonia	22 (61%)	8 (57%)	14 (64%)
Sinusitis	18 (50%)	8 (57%)	10(45%)
Otitis	16 (44%)	7 (50%)	9(41%)
Chronic diarrhea	5 (14%)	1 (7%)	4 (18%)
Gastroenteritis	4 (11%)	1(7%)	3 (14%)
Lymphoma	3(8%)	0(0%)	3 (14%)
Bronchiectasis	4(11%)	0 (0%)	4 (18%)
Splenomegaly	7 (19%)	3 (21%)	4 (18%)
Lymphadenopathy	5 (14%)	0 (0%)	5 (23%)
Granulomas	2 (6%)	1 (7%)	1 (5%)
Mean IgG (mg/dL)	397	412	387
Mean IgA (mg/dL)	173	76	230
Mean IgM (mg/dL)	55	44	62
Freiburg's classification			
Ia	8	5	3
Ib	20	6	14
II	8	5	3

Treg (FOXP3+) compared to healthy controls (p= 0.0002, p= 0.0005, respectively). Patients with autoimmunity have more innate CD21 B cells than patients without autoimmunity (p= 0.0075, p= 0.0488). There is also a correlation between increased of CD4+ CD45RO T cells and less Treg (r= -0.5077 p= 0.0069) (Figure 1).

Conclusions: These studies suggest that the development of autoimmunity in CVID patients relates to both restricted numbers of B cells and restricted help from T cells. The profound immune dysregulation in CVID patients may also cause expansion of autoimmune B cell clones. The finding of high incidence of CD21low B cells are significant as these cells have been found to be enriched for autoreactive germline antibodies. The finding of a higher proportion of memory T cells in CVID patients with autoimmune diseases suggests a lack of replenishment of the CD4 T cell pool by

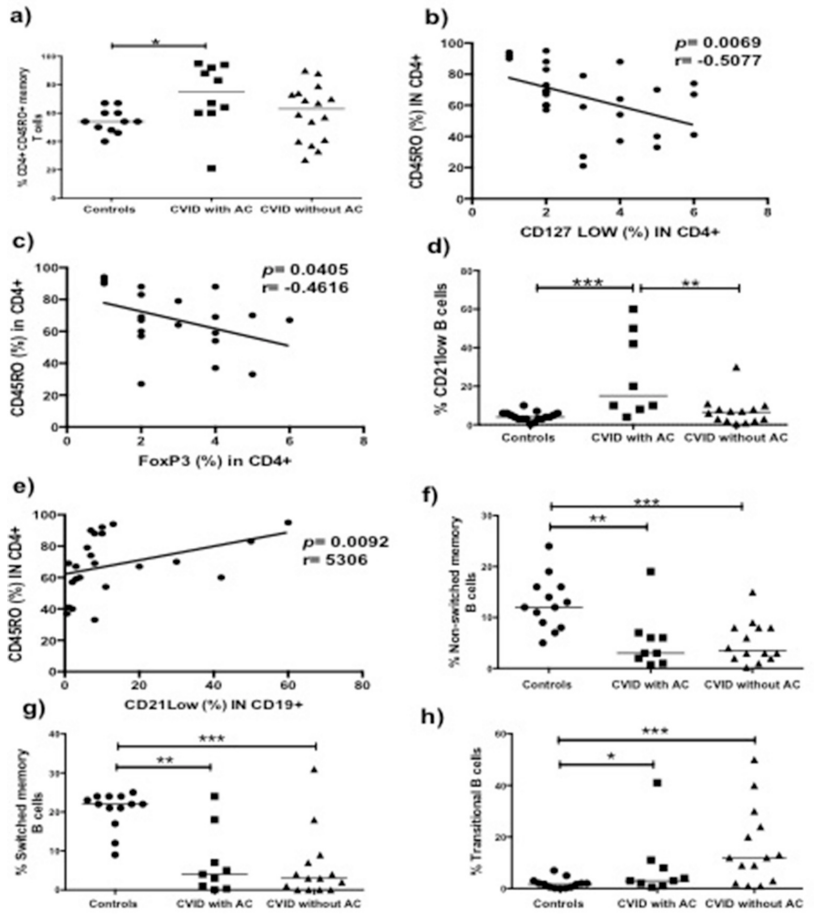


FIGURE 1: Analysis of T and B subpopulation. Flow cytometric analysis of T and B supopulation percentage from CVID patients with autoimmune diseases (AD) (n=l4)(■); CVID patients without AD (n=22) (▲); these groups were compared with controls (n= 13) (●). a) CD4+ CD45RO+T cells percentage; b) correlation analyses between CD4+ CD45RO+ T Cells and Treg CD127 low cells; c) correlation analyses between CD4+ CD45RO+ T cells and Treg FoxP3+ cells; d) CD21 low B cells (CD19+ CD21- CD38-); e) correlation analyses between CD4+ CD45RO+ T cells and CD21 low B cells (CD19+ CD21- CD38-); f) non-switched memory B Cells (CD19+ CD27+ IgD+); g) switched memory B cells (CD19+ CD27+ IgD-); h) transitional B cell (CD19+ CD24+++CD38+++). Differences between patients and controls were compated using Mann-Whitney U-test. (*) significant, p=<0.05; (**) very significant, p=< 0.01; and (***) highly significant, p= < 0.001. AC= Autoimmune cytopenia.

new thymic-derived cells. These observations suggest that the coexistence of autoimmunity and immunodeficiency is a result of dysfunction in multiple immune pathways.

REFERENCES

1. Arandi N, Mirshafiey A, Abolhassani H, Jeddi-Tehrani M, Edalat R, Sadeghi B, et al. Frequency and expression of inhibitory markers of CD4(+) CD25(+) FOXP3(+) regulatory T cells in patients with common variable immunodeficiency. Scand J Immunol. 2013;77(5):405-12.
2. Fevang B, Yndestad A, Sandberg WJ, Holm AM, Muller F, Aukrust P, et al. Low numbers of regulatory T cells in common variable immunodeficiency: association with chronic inflammation in vivo. Clinical and experimental immunology. 2007;147(3):521-
3. Horn J, Manguiat A, Berglund LJ, Knerr V, Tahami F, Grimbacher B, et al. Decrease in phenotypic regulatory T cells in subsets of patients with common variable immunodeficiency. Clinical and experimental immunology. 2009;156(3):446-54.
4. Gathmann B, Mahlaoui N, Ceredih, Gerard L, Oksenhendler E, Warnatz K, et al. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. The Journal of allergy and clinical immunology. 2014;134(1):116-26.
5. Mouillot G, Carmagnat M, Gerard L, Garnier JL, Fieschi C, Vince N, et al. B-cell and T-cell phenotypes in CVID patients correlate with the clinical phenotype of the disease. Journal of clinical immunology. 2010;30(5):746-55.
6. Wehr C, Eibel H, Masilamani M, Illges H, Schlesier M, Peter HH, et al. A new CD21low B cell population in the peripheral blood of patients with SLE. Clinical immunology. 2004;113(2):161-71.
7. Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002;99(5):1544-51.

Experience in the treatment of the refractory condylomatosis with interferon recombinant alfa2 in Cienfuegos hospital

Alain Alonso Remedios

Medical University of Cienfuegos, Cienfuegos, Cuba
alain.alonso@gal.sld.cu

Keywords: interferon, cost, Adverse event, Human papiloma virus, genitalswarts

Introduction: Therecombinat interferón alpha 2 isreservedonlytorefractary condilomatosis infections, becauseit has highcost and several adverse events.

Objetive: Compararingtheclinical response totherecombinat interferón alpha 2 in pacientswhitrefractaryteatriesbysystemic and intralesional choice.

Método: Theyincluded 47 patientsthattheyshared in twogroups. One of themsrecived 3 millionunit of interferón 3 time per weekforsystemicway (n=26); Whiletheotheronereceived 5 millionunit intralesional once per week (n=21). Bothgroupswereevaluatedby 12 weeks. Thestudied variables were:age, sex,occupation, schoollevel, civil state, time of evolution, number of relapses, number and size of warts, response tothetreatment, adverse effects and cost of thetreatment.

Resultados: Thefeminime sex predominated and theMiddleAgeswas of 24.9 yearsold. Mostpatientswere student and single.Mean time of evolutionwas of 11.7 monthswith 2.6 recidives. Los pacientes presentaban 7,4 lesiones como promedio con un tamaño de 3,5 mm.Thepatientshad a mean of 7.4 wartswithsize of 3.5 mm.Whitbothtreatment. Withbothtreatmentwas a significantreduction of thenumber and size of thewarts, notexistingstatisticallydifferencebetween intralesional and systemicadministrations (p=0,34). Themostfrequent adverse effects in bothgroupswerecephalea, theelevation of temperature, thesleepiness and theasthenia; it´sexperimented a decrease of theintensity in time. Thetreatmentdidnotmodifythehematological variables and transaminases. Thetreatment intralesional achieved a saving of 30.72 pesos forpatient in relationtothesystemic.

Conclusiones: Differenceswerenotfound in the response tothetreatmentneither in the adverse events in bothroutes ofadministration of theinterferon; beingthe intralesional treatment more economicforthe hospital tan systemictreatment.

Characterization of nodular lymphoid hyperplasia in children with food allergy

Boris Luis Fernandez de la Cruz[1], Maytee Mateo Morejón[2], María Viviana Paullán[3], Elsa Garcia[3], Deyanira La rosa[3], Teddy Osmin Tamargo Barbeito[4]

[1]Pediatric Hospital of the hill, La Habana, Cuba
[2]Centro Nacional de Biopreparados, Havana, Cuba
[3]Instituto de Gastroenterologia (Cuba), La Havana, Cuba
[4]Hermanos Ameijeiras Hospital, Havana, Cuba
borislfc82@gmail.com; mayteemm@biocen.cu; maria@infomed.sld.cu; elsa@infomed.sld.cu; deyaniri@infomed.sld.cu; teosmin@infomed.sld.cu

Keywords: Food, allergy, Characterization, Nodular lymphoid hyperplasia, Children

Rationale: Gastrointestinal lymphoid nodular hyperplasia (NHL) is uncommon between 7 and 18 years, has been associated with food allergies, giardiasis, humoral immunodeficiencies among other diseases. Hypothesis: To determine if the clinical, immunological and histological characteristics of pediatric patients with HNL in Cuba are related to food allergy.

Methods: case series, descriptive, cross-sectional. Between July 2014 and January 2016. In pediatric patients with diagnosis of lymphoid hyperplasia of the colon and / or terminal ileum, suspected by endoscopy and confirmed histologically. Goldman's criteria were used to determine the relationship between the onset of digestive symptoms and exposure to a given food. The prick test (PT) was performed with standardized extracts. Total immunoglobulins, C-reactive protein, rheumatoid factor and sensitization to allergenic extracts were determined by PT tests, specific IgE and IgG4 identification, using the western blotting technique.

Results: 50 patients were enrolled. The ages 7–10 prevailed. All patients had a history of personal and family atopy. The PT was positive in 60%, of them 68% had HNL located in the ileum, in Western blotting of IgE and IgG4 coincided in sensitivity with the PT, the predominant band was Bos d 8 for cow's milk, food of higher incidence (75.0%) Rectal bleeding was present in 62.0% of patients. More than 80.0% presented normal values of immunoglobulins, PCR, FR and 60.0% were related to giardiasis.

Conclusions: The frequency of HNL was observed in more than half of patients with PT positive. Further research is needed to confirm whether there is a link between HNL and food allergy.

Curricular strategies for to handle current challenges in the postgraduate medical education of the immunology specialty in Cuba

Carlos Agustín Villegas Valverde

[1]*Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba*
[2]*Instituto Nacional De Oncología Y Radiobiología (INOR), La Habana, Cuba*
carlosvillega@infomed.sld.cu

Keywords: immunology, Medical Education, Challenges, Curriculum Design, Residency program

Introduction: Immunology in Cuba is a science and, at the same time, a field of medicine and teaching in several university careers. In the case of postgraduate education in medical sciences is a specialty classified within the basic biomedical sciences, however, has the peculiarity of having a broad student's exit profile. Its graduates can insert themselves in different scenarios inside and outside of public health.

In the medical career, the immunology is taught within the undergraduate training in an integrated manner in several subjects and since the present course, it was introduced as an independent subject. In addition, the vertical internship in immunology was created. Specialist in immunology with its contents and skills contribute to continued education toward masters and doctors of science.

In the last three decades the volume of knowledge of immunology has grown dramatically, which it is evident by the growing number of scientist journals in immunology and publications related to it. This fact was enhanced by the emergence of the HIV epidemic, which in turn allowed deepening the understanding of the functioning of the immune system in health and disease. The enormous, dynamic, complex and complicated knowledge framework of immunology is a great challenge for those who decide to study and teach it. However, there are few publications aimed at addressing the challenges of teaching this discipline in medical sciences. To know the challenges that faces any discipline, allow to design a curricular strategy that solve them properly.

This work is aimed at examining the contribution of the Cuban curriculum of the immunology residency to confront their current challenges.

Table 1: Challenges and curricular strategies to solve the challenges of teaching immunology in Cuba.

Challenges	Curriculum strategy
Development of computer and computing.	Implementation of computer curricular courses and systematic use of bibliographic searches and information search designs.
High rate of knowledge production.	Developing skills in self-education students and guaranteeing an adequate time fund of independent study. Developing skills of directed search of essential information and critical review of content. Developing skills in facilitating learning rather than in teaching by professors.
High applicants number	Decentralizing the accredited centers to teach in a territorial way and distributing the students by different teaching scenarios according to rotations scheduler.
Increase in the number of universities and diversity of training curricula.	Defining a university and a guiding center in the training of the immunology specialist, without limiting the training spaces to this center. Application of a unique training curriculum to the whole country created and updated by an experts team.
Privatization of universities.	Cuban universities are state-run and respond appropriately to growing social needs.
Fairness and accessibility to education.	All medical graduates have the right and duty to continue their postgraduate studies, but there is a system of competition and requirements for applying to the specialty, which respond to its characteristics and the needs of the country in this field.

New challenges in university education are included in the immunology postgraduate education.

The pre and postgraduate teaching in universities is facing a set of new challenges, arising from the development of science and the needs of society as a whole. The meaning of new refers to current, since they have been evidenced in recent decades, but the university has not fully produced the qualitative leap that is required, to successfully overcome them. Within these, there are:

- Development of computer science.
- The development of information and communication technologies.

- High rate of knowledge production.
- Overcrowding of the university registrations.
- Increase in the number of universities.
- Increase in the number and diversity of curricula.
- Privatization of universities.
- Equity and accessibility to higher education.

In the immunology specialty curriculum, strategies were designed that took into account the current challenges of medical education. All of these challenges are found in postgraduate education too and are an indissoluble part of higher education. In addition to this problem the immunology have its own teaching challenges such as:

- Very complex and complicated contents.
- Rapid obsoleteness of the knowledge.
- Eradicate the predominance of the theoretical and descriptive form of contents teaching.
- Very expensive cost of the experimentation. (Immunology laboratory conditions, technology and reagents)
- Technology with great complexity.
- The character of assay of many experiments.
- Clinical application of immunology.

In current 2017–2018 academic year, was started the new training program of the immunology specialist, which has a teaching strategy that also includes the current challenges of medical education. Structure of the syllabus is an eminently modular, which allows flexibility in the grouping and sequence of the contents and gives the possibility of partial certifications of the students.

The major challenge in the teaching of immunology for four years, is to condense the decades accumulation of a broad knowledge and apply them to medical practice. Especially because this science stopped being descriptive to be widely applied, reaching the genomic and molecular levels.

However, these are not the only challenges facing the teaching of immunology because as a field of knowledge has its own. In table 2, you can appreciate the strategies adopted in the curriculum to face these types of challenges.

The transformations that immunology has undergone in its basic conception have been decisive for its evolution. It has gone from a branch of science to the service of microbiology and infectology to be a totally independent science, leaving the paradigms of combat and defense for dynamic homeostasis.

Table 2: Challenges and curricular strategies to solve the challenges of immunology as a medical specialty.

Challenges	**Curriculum strategy**
Very complex and complicated contents.	New structure and flexibility of logical order of the contents to be taught and use of conceptual models. Development of self-study and investigative aptitude.
Rapid obsoleteness of the knowledge.	Diversifying and updating the bibliography systematically. Develop scientific attitude and collective learning strategies. Using IT properly.
To eliminate the predominance of the theoretical and descriptive form of contents teaching.	To enhance problem solving based strategies, provide algorithms and model for solution, engage robust discussion of real clinical cases, to demonstrate the utility of the theories in a clinical context. Using textbooks with interactive online version.
Technology with great complexity.	To begin with laboratory practice since first year of residence.
Very expensive cost of the experimentation. (Immunology laboratory conditions, technology and reagents)	Make rotations for prestigious and accredited research centers and institutes that have higher technologies and teacher with wide expertise
The character of assay of many experiments.	Begin with assay that have high grade of standardization and validation process that permits a strong reproducibility.
Clinical application of immunology.	Employing concepts and technology from translational basic research to clinical applications. To insert the residents in a medical team in every hospital and rotation that allows it.

Looking to the future, we must include mathematical modeling and computational models in the training programs to simulate and predict the cellular and molecular interactions of the immune system.

A professional trained under these precepts may be in a better position to face the new challenges posed to immunology such as stopping aging, treating chronic diseases such

as cancer, diabetes or dementias, developing tolerance to transplants and treatment of human reproduction problems as the infertility.

Conclusions: The creation in Cuba of a new training plan for the medical specialist in immunology, with a curricular design that allows for the optimal development of professional competencies, including strategies that address the current challenges of postgraduate medical education, prepares the specialist to address their Once again, the new and enormous challenges that immunology presents as a science today.

Evaluation of plasticity features in pathogenic Th17 lymphocytes from the blood and skin of psoriasis patients

Cristina Nayeli Aguilar-Flores[1,2], Octavio Castro-Escamilla[2,3], Vadim Perez-Koldenkova[2], Laura Bonifaz[2]

[1]Instituto Politécnico Nacional, Mexico City, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
acristins@outlook.com; larenmc@msn.com; lnma.cmn.sigloxxi@gmail.com; labonifaz@yahoo.com

Keywords: Psoriasis, plasticity, RORγt, Th17 lymphocytes, Pathogenic Th17 Lymphocytes

Background: Psoriasis is a skin disease characterized by erythema and the formation of raised skin plaques. Because of the discovery of IFN-γ in peripheral blood (PB) and cell cultures of lesional skin, it was thought that Th1 lymphocytes were a crucial mediator in the disease course. However, with the identification of IL-17 and IL-22 in PB and dermis of psoriatic patients, it has been proposed the Th17 lymphocytes as the most important participant in the development of the disease. Recently, a new pathogenic phenotype of Th17 lymphocytes has been described in animal EAE models and human pathologies. Pathogenic Th17 lymphocytes are characterized by the production of low quantities of IL-17 and IL-22 but high expression of IFN-γ. Additionally the transcriptional signature of this phenotype includes the co-expression of ROR-γt and T-bet. Furthermore, new evidence shows plasticity capabilities of Th17 lymphocytes towards a Th1 profile. These subsets are characterized by preserving CD161, fully acquiring T-bet and expressing IFN-γ only. This Th17 plasticity has been associated to the exacerbation and severity of some inflammatory diseases. Staphylococcus bacteria have been reported to be one of the most numerous members in the skin microbiome and could potentially be involved in the progression of psoriatic lesions. Previous results from our group demonstrate that Th17 lymphocytes obtained from psoriatic lesional dermis cultures, acquire pathogenic features after activation with Staphylococcus aureus enterotoxin-B (SEB). The pathogenic features include the increased expression of ROR-γt, T-bet lack of IL-17 but high expression of IFN-γ. Importantly, CD4+ lymphocytes producers of IL-17, IL-17 and IFN-γ, and IFN-γ were found in lesional psoriatic skin. These results might suggest plasticity capabilities in the Th17 lymphocytes from psoriasis patients. However it remains unknown if this phenomenon occurs in PB and lesional skin of psoriasis patients and if it is related to the severity of the disease.

Aim: To determine the existence, phenotype, cytokine profile and plasticity features of peripheral blood pathogenic Th17 lymphocytes from psoriasis patients. To determine and quantify the existence of pathogenic Th17 cells in situ and correlate it with the severity of the disease.

Method: PBMCs were obtained from psoriasis patients and healthy controls. Next they were activated with SEB or antibodies anti-CD3 and anti-CD28 for 72h. Production of cytokines IL-17 and IFN-γ, expression of CD161 and transcription factors T-bet and ROR-γt were assessed with flow cytometry analysis in the T lymphocyte CD4+ROR-γt population. To evaluate and quantify the expression of IL-17, IL-17/IFNγ and IFN-γ in the T lymphocyte CD4+ population in situ, biopsies from lesional and non lesional skin of psoriasis patients were included in paraffin blocks, next immunofluorescence and confocal microscopy assays were performed.

Results: SEB stimulation on PBMCs of psoriasis patients showed an increment in the percentage of cells expressing ROR-γt in CD4+ lymphocytes. Additionally this ROR-γt+CD4+ (Th17) population co-produces IL-17 and IFN-γ. Next we activated PBMCs of psoriasis patients with Anti-CD3 and anti-CD28 in order to asses if another TCR stimuli could induce the same results. CD4+ cells activated with CD3/CD28 maintained CD161 expression, increased the percentage of cells expressing ROR-γt, and induced IL-17 and IFN-g production in a similar way as SEB stimulation did. Thus, we decided to use CD3/CD28 stimuli for the development of this research. Furthermore, these activated Th17 lymphocytes (CD4+RORγt+CD161+) from patients, produced IL-17 and IFN-γ in three different patterns: production of IL-17 only, co-expression of IL-17 and IFN-γ and IFN-γ only. On the other hand the activation of PBMCs from healthy controls with this stimuli induced an increment in the percentage of CD4+ cells expressing ROR-γt. These Th17 lymphocytes produced low percentages of IL-17 and IFN-γ. Nevertheless, these patterns of cytokines production in patients and healthy controls are independent to the expression of T-bet and ROR-γt. We sought to determine the existence of Th17 cells and their cytokine expression in situ. Immunofluorescence assays in lesional and non-lesional skin were standardized and are currently being performed.

Conclusions: Our results show the existence of two populations of pathogenic Th17 lymphocytes in PB from psoriasis patients. One is characterized by the production of both IL-17 and IFN-γ, which could be the result of plasticity of Th17 lymphocytes

towards a Th1 phenotype. Whereas the other population produces IFN-γ only, which could suggest that Th17 cells have fully acquired a Th1 lymphocyte program. These findings in PB may be related to the severity of the disease and could be considered for new biological strategy treatments.

Contribution of HIV patient serum antibodies that recognize lymphocyte antigens to the inhibition of the HIV-1 envelope-dependent membrane fusion

Mirna Berenice Ruiz Rivera*[1], Guillermo Gómez Icazbalceta*[1], Marcela Vélez Alavez[1], Mónica Viveros Rogel[2], Santiago Pérez Patrigeon[2], Edmundo Lamoyi[1], Luis Llorente Peters[2], Carlos A Núñez-Álvarez[2], Leonor Huerta Hernández[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
mirbere@gmail.com; ggicazbalceta@gmail.com; marcela.velez.alavez@gmail.com; moviveros@gmail.com; santiago.perez@infecto.mx; lamoyi@biomedicas.unam.mx; luisllorentepeters57@gmail.com; nuac80df@yahoo.com; leonorhh@biomedicas.unam.mx

Keywords: Autoantibodies, HIV, Membrane Fusion, Viral Load, IgG, IgM, Anti-lymphocyte antibodies, Virus fusion

Background: A feature of highly pathogenic HIV-1 virus strains is an increased ability to induce membrane fusion, the event mediating the entry of virus to target cells [1,2]. Increased fusion capacity promotes infection of target cells by free virus, as well as the transmission of cell-associated virus from infected to uninfected cells through virological synapses [3,4], the formation of infected multinucleated cells [5], and lymphocyte apoptosis [6]. Besides the interaction of the HIV fusion protein (Env) with CD4 and chemokine receptors, the efficiency of HIV-1-entry is increased by receptor/signaling cellular surface molecules incorporated into the virus membrane [7]. Anti-CD4 antibodies present in the circulation of HIV-1 infected individuals have been associated with protection [8]; however, antibodies against other cell-associated molecules have been also detected during HIV-1 infection [9], and they may participate in the inhibition of Env-mediated fusion processes. Autoantibodies recognizing lymphocyte membrane molecules[10] may participate in modulation of the Env-mediated membrane fusion by binding to the infected or the target cells, or to cellular constituents exposed on the virus membrane.

Objetive: Here we assessed the contribution of anti-lymphocyte antibodies (ALA) other than anti-CD4 antibodies, to the inhibition of the HIV-1 Env-mediated lymphocyte membrane fusion by testing the effect of 38 sera from HIV-1 infected men and 30 healthy control individuals, before and after adsorption of sera on Jurkat CD4– cells.

Material and Methods: Human Jurkat lymphocytic T cells expressing a subtype B HIV-1 envelope (Env+ cells) and CD4+ Jurkat cells were differentially labeled with lipophilic probes (fluorescent in red and green upon laser excitation), and co-cultivated for several hours to allow cell-cell fusion. In this assay fused cells are readily detected as double fluorescent particles by flow cytometry [1–5]. To determine if ALA from HIV-1 infected patients are able to influence the HIV Env-mediated cell fusion, we determined the effect of sera on fusion before and after absorption on CD4– Jurkat cells. CD4– Jurkat cells were used in order to avoid the influence of virus particles and virus-antibody immune complexes in any change of the effect of sera after absorption. This process also favored the assessment of participation of ALA with specificities that differ from CD4 in fusion inhibition. The change of activity of sera on fusion after absorption was compared with the level of binding of serum IgG and IgM to the Jurkat CD4– cells, the viral load, and the CD4 cell count. Sera from 38 HIV-positive patients (treatment-naïve men) and 30 healthy donors were tested.

Results: Adsorption of HIV-1-positive sera on CD4-negative Jurkat cells produced three different effects on the fusion activity of HIV+ sera. After absorption, 58% of sera exhibited a decreased fusion inhibition capacity respective to the same non-absorbed sample. A mean decrease of 33% of the fusion inhibition activity was removed by absorption in this group. The opposite effect was observed in 31.6% of sera, which showed a greater fusion inhibition after absorption with a mean 31% difference respective to the non-absorbed sample. Finally, absorption did not modify the effect of sera in 10.5% of cases. HIV-positive sera contained IgG and IgM able to recognize the surface of Jurkat CD4– cells, and absorption on Jurkat CD4– cells removed significantly this activity. Patient's viral load was significantly higher in sera non-containing than in sera containing ALA-related fusion inhibition activity.

Conclusions: These observations suggest that serum ALA, other than anti-CD4 antibodies, contribute significantly to inhibition or promotion of the HIV Env-mediated membrane fusion. ALA-related fusion inhibition (ALA-rFI) activity may prevail in about 58% of HIV+ individuals, whereas ALA-related fusion enhancing (ALA-rFE) activity may predominate in one third of this population. Viral load was significantly lower in sera containing ALA-rFI activity, suggesting that autoantibodies recognizing the lymphocyte surface may participate in virus containment during HIV-1 infection. Thus, the inhibitory effect of HIV-envelope mediated cell-cell fusion by antibodies from HIV-1 infected patients would be due to the combined activity of antibodies against the HIV envelope and against lymphocyte antigens.

REFERENCES

1. Blumenthal, R., Durell, S., Viard, M. (2012) HIV entry and envelope glycoprotein-mediated fusion. The Journal of biological chemistry 287, 40841-40849.
2. Blaak, H., van't Wout, A. B., Brouwer, M., Hooibrink, B., Hovenkamp, E., Schuitemaker, H. (2000) In vivo HIV-1 infection of CD45RA(+)CD4(+) T cells is established primarily by syncytium-inducing variants and correlates with the rate of CD4(+) T cell decline. Proceedings of the National Academy of Sciences of the United States of America 97, 1269-1274.
3. Chen, P., Hubner, W., Spinelli, M. A., Chen, B. K. (2007) Predominant mode of human immunodeficiency virus transfer between T cells is mediated by sustained Env-dependent neutralization-resistant virological synapses. Journal of virology 81, 12582-12595.
4. Jolly, C., Mitar, I., Sattentau, Q. J. (2007) Adhesion molecule interactions facilitate human immunodeficiency virus type 1-induced virological synapse formation between T cells. Journal of virology 81, 13916-13921.
5. Symeonides, M., Murooka, T. T., Bellfy, L. N., Roy, N. H., Mempel, T. R., Thali, M. (2015) HIV-1-Induced Small T Cell Syncytia Can Transfer Virus Particles to Target Cells through Transient Contacts. Viruses 7, 6590-6603.
6. Joshi, A., Nyakeriga, A. M., Ravi, R., Garg, H. (2011) HIV ENV glycoprotein-mediated bystander apoptosis depends on expression of the CCR5 co-receptor at the cell surface and ENV fusogenic activity. The Journal of biological chemistry 286, 36404-36413.
7. Chertova, E., Chertov, O., Coren, L. V., Roser, J. D., Trubey, C. M., Bess, J. W., Jr., Sowder, R. C., 2nd, Barsov, E., Hood, B. L., Fisher, R. J., Nagashima, K., Conrads, T. P., Veenstra, T. D., Lifson, J. D., Ott, D. E. (2006) Proteomic and biochemical analysis of purified human immunodeficiency virus type 1 produced from infected monocyte-derived macrophages. Journal of virology 80, 9039-9052.
8. Burastero, S. E., Figini, M., Frigerio, B., Lusso, P., Mollica, L., Lopalco, L. (2009) Protective versus pathogenic anti-CD4 immunity: insights from the study of natural resistance to HIV infection. Journal of translational medicine 7, 101.
9. Zandman-Goddard, G. and Shoenfeld, Y. (2002) HIV and autoimmunity. Autoimmun Rev 1, 329-337.
10. Dorsett, B. H., Cronin, W., Ioachim, H. L. (1990) Presence and prognostic significance of antilymphocyte antibodies in symptomatic and asymptomatic human immunodeficiency virus infection. Arch Intern Med 150, 1025-1028.
11. Martinez-Mendez, D., Rivera-Toledo, E., Ortega, E., Licona-Limon, I., Huerta, L. (2017) Monocyte-lymphocyte fusion induced by the HIV-1 envelope generates functional heterokaryons with an activated monocyte-like phenotype. Exp Cell Res 352, 9-19.
12. Huerta, L., Lamoyi, E., Baez-Saldana, A., Larralde, C. (2002) Human immunodeficiency virus envelope-dependent cell-cell fusion: A quantitative fluorescence cytometric assay. Cytometry 47, 100-106.
13. Huerta, L., Lopez-Balderas, N., Larralde, C., Lamoyi, E. (2006) Discriminating in vitro cell fusion from cell aggregation by flow cytometry combined with fluorescence resonance energy transfer. Journal of Virological Methods 138, 17-23.
14. Gomez-Icazbalceta, G., Ruiz-Rivera, M. B., Lamoyi, E., Huerta, L. (2015) FRET in the Analysis of In Vitro Cell-Cell Fusion by Flow Cytometry. Methods in Molecular Biology 1313, 217-227.
15. Huerta, L., Gomez-Icazbalceta, G., Soto-Ramirez, L., Viveros-Rogel, M., Rodriguez, R., Fuentes, L., Lamoyi, E., Larralde, C. (2005) Human immunodeficiency virus 1 (HIV-1) envelope-dependent cell-cell fusion modulation by HIV-positive sera is related to disease progression. Journal of General Virology 86, 1961-1966.

Hydroxypropyl-beta-cyclodextrin as a potential drug to control chronic inflammation in HIV infection by inhibiting the activation of myeloid cells by microbial products

Flavio Lemos Matassoli[1,2], Ihid Carneiro Leao[1], Bruno Braz Bezerra[2], James Hildreth[1,3], Luciana Barros Arruda[2]

[1]University of California, Davis, Davis, United States
[2]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[3]Meharry Medical College, Nashville, United States
flaviomatassoli@hotmail.com; ihidleao@gmail.com; brunobrazb@gmail.com; jhildreth@mmc.edu; arruda@micro.ufrj.br

Keywords: HIV, tNF-alpha, Immunoregulation effect, BCD, Monocyte activation

Chronic immune activation is a hallmark of HIV infection, which results from the activation of lymphocytes and monocytes by the virus or by exposure to bacterial products due to microbial translocation. Monocytes derived from HIV patients are highly activated and produce enhanced TNF-alpha upon stimulation with bacterial lipopolysaccharides (LPS). Elevated TNF-alpha levels have been described as a major issue related to impaired CD4 control during chronic viral infections, including HIV. Increased levels of plasma IL-10 were also correlated to higher viral loads and lower CD4 T cell counts in HIV patients. Therefore, the development of new therapeutic strategies to regulate the enhanced inflammatory status besides controlling viral load should be beneficial to HIV patients.

Hydroxypropyl -beta-cyclodextrin (HP-B-CD) is a cholesterol-sequestering drug, which has been previously suggested as an anti-HIV drug due to its ability to inactivate HIV in vitro, and to reduce HIV infectivity in a primate experimental infection. Interestingly, antigen-presenting cells (APC) derived from HIV-infected non-progressors (NP) patients presented lower levels of membrane cholesterol than cells from HIV progressors. Also, the expression levels of the lipid uptake receptor CD36 were negatively correlated CD4 T cells counts in HIV+ patients. Also, there is increasing evidence that cholesterol metabolism may be associated to the regulation of different transduction signals associated to cytokine production and inflammation. In this sense, IFN-induced accumulation of 25-hydroxycholesterol resulted in the inhibition of IL-1beta secretion by activated macrophages and downregulated the de novo synthesis of cholesterol, indicating that lower levels of membrane cholesterol and

accumulation of intracellular cholesterol intermediates may regulate the inflammatory response.

Therefore, we hypothesized that treatment of HIV+ patients with HP-B-CD could delay HIV progression by both inhibiting virus replication and HIV-associated inflammatory response. To investigate this hypothesis, we treated myeloid cells derived from HIV positive and HIV negative donors with HP-B-CD and addressed whether it would impact the inflammatory status of those cells induced by LPS, as a surrogate of microbial stimulation.

Initially, we determined the nontoxic concentration of HP-B-CD and evaluated the kinetic of cholesterol depletion in primary monocytes. Cells were treated with different concentrations of HP-B-CD for 1hour, then the medium was substituted by complete medium and the cells were cultured for further 48h. Treatment with 10mM HP-B-CD reduced about 60% of the membrane cholesterol content after 1h treatment, with 80% recovery after 48h culture; whereas 1mM HP-B-CD did not significantly affect cholesterol levels at the evaluated time points. After 48h of HP-B-CD treatment the intracellular concentration of sterol intermediates, oxysterols and sitosterols were similar to nontreated cells and no obvious alteration in cholesterol cellular distribution was observed. Importantly, raft disruption was also reestablished at this time point, since we did not detect any alteration in the expression of CD59 and CD45, evaluated by flow cytometry, as raft and non-raft markers.

To investigate the effect of HP-B-CD in monocytes activation, the cells were treated or not with BCD and stimulated with LPS after 48h (after membrane cholesterol and raft distribution recover). Then, the expression of the lipid receptor CD36 and of TNF- were evaluated by flow cytometry. Monocyte treatment with HP-B-CD significantly reduced LPS-induced CD36 and intracellular TNF- expression, even at very low drug concentration, suggesting that cholesterol depletion, but also other undetermined mechanisms, might impair the activation of monocytes. Corroborating with these results, the secretion of TNF- induced by LPS was significantly decreased in the cells pretreated with HP-B-CD. Interestingly, IL-10 secretion was also strongly reduced, but the secreted levels of several other cytokines were unaffected, including IL-1beta, IL-1RA, IL-2, IL-2R, IL-4, IL-7, IL-12, IL-13, IP-10, MIG-1, IFN-alpha and IFN-gamma, demonstrating that HP-B-CD effect was not associated with general monocyte inactivation. Treatment with monocytes with 1mM HP-B-CD induced a similar effect of 10mM, suporting the idea that membrane cholesterol depletion is not the sole mechanism leading to the inhibition of LPS-induced activation.

We then evaluated whether HP-B-CD treatment would modulate the expression of those cytokines by measuring TNF-alpha and IL-10 mRNA levels by qRT-PCR. As

expected, stimulation of monocytes with LPS induced the expression of TNF- and IL-10 mRNAs. However, the expression of both cytokines was significantly reduced in the cells pretreated with HP-B-CD, indicating that the drug impaired LPS-induced inflammatory response by decreasing cytokine transcription.

TLR4 activation by LPS trigger distinct signal transduction pathways associated to cytokine secretion, including the activation of PI3K and MAPK. Therefore, we start to evaluate which pathway would be inhibited by HP-B-CD, impairing the monocyte response to LPS. We observed that monocytes treated with HP-B-CD did not increase the expression of phosphorylated p38 upon LPS activation, as evaluated by western blotting. On the other hand, activation of Erk MAPK or PI3K were not significantly affected by HP-B-CD. These data suggest that the diminished response to LPS induced by HP-B-CD resulted from the inhibition of specific signaling pathways, which may explain the regulation of TNF- and IL-10 production, but not other cytokines.

We finally addressed the effect of HP-B-CD on the expression of monocytes activation markers associated to stimulation of specific lymphocytes. Since increased expression of MHCII and co-stimulatory molecules are induced by LPS and are detected in chronic activated monocytes, we addressed the expression of HLA-DR and CD86 in the monocytes treated with HP-B-CD by flow cytometry. Our data demonstrated that pretreatment of monocytes with the drug reduced the expression of both molecules after cell activation with LPS. In addition, PBMC treatment with HP-B-CD decreased the surface expression of HLA-DR in CD14-negative BDCA1+ and BDCA3+ cells, which are subpopulations of myeloid DCs. The latter data suggest that HP-B-CD may also affect the competence of bacteria-stimulated myeloid cells to activate T cells.

Taken together, our findings demonstrated that HP-B-CD may contribute to down-regulate HIV-associated chronic immune activation, besides its well-known ability to inhibit viral replication, being worthy, therefore, to be further investigated as a potential anti-HIV drug.

Characterization of the cellular components in peripheral blood aggregates from patients diagnosed with systemic inflammation

Jessica Lakshmi Prieto-Chavez[1], Juan Alberto Hernández-Aceves[1], Graciela Libier Cabrera Rivera[1], Lorena Hernández Torres[1], Arturo Cérbulo-Vázquez[2], Vadim Pérez Koldenkova[1], Eduardo Antonio Ferat-Osorio[1], Armando Isibasi[1], Lourdes Andrea Arriaga-Pizano[1]

[1]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Combined Medicine Study Plan, Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico
lakshmi.litmus@hotmail.com; dragg412@gmail.com; libier_9@hotmail.com; lorenfq.02@hotmail.com; cerbulo@hotmail.com; vad.perez@gmail.com; eduardoferat@prodigy.net.mx; isibasi@prodigy.net.mx; landapi@hotmail.com

Keywords: Neutrophils, Platelet Activation, Sepsis, Systemic Inflammatory Response Syndrome, cellular aggegates

The systemic inflammatory response come to place when a localized inflammatory response cannot be contained, which favors the mass release of inflammatory mediators into the circulatory system in the face of abrupt events such as infections, traumatisms or extensive burns. When systemic inflammation is associated with an infection (and with failure of at least one organ) it is called sepsis. Sepsis has mortality rates from 20 to 60% (depending on the severity of the condition), which makes it a serious health problem in the Intensive Care Units (ICU) worldwide [1]. These inflammatory processes are characterized by exacerbated increase in production of pro- and anti-inflammatory cytokines (IL-6, IL-8, TNF-α, IL-10, TGF-β), this deregulates the immune system as well as the coagulation, complement and endothelial response systems. One of the most severe complications in sepsis is disseminated intravascular coagulation, which is directly related to endothelial and platelet activation. Increased expression of adhesion molecules in leukocytes and activation markers (e.g.: CD69, CCR7, ICAM-1) has been reported in both cell models and patients with sepsis. Recent work made in our laboratory showed that blood cells of septic patients present increased frequency of detectable cell aggregates by multiparametric flow cytometry, compared with healthy subjects. Neutrophils resulted to be the most frequent cells involved in these aggregates. However, we do not know if there are other key blood elements in the formation of the aggregates [2]. Several factors can determine this phenomenon, like formation of neutrophil extracellular traps (NETs), activation of the coagulation pathways or cell activation itself (endothelial cells). Koji Tanaka and col. have suggested that NETs take part in the generation of cellular aggregates, with DNA being the main mediator of

the union between leukocytes [3]. While Gawaz et. al. have suggested that activated platelets may favor leukocyte aggregation[4], which matches Rondina and collaborator's report of 2015 where they describe an increased formation of leukocyte-leukocyte and platelet-leukocyte aggregates in patients with sepsis compared to healthy volunteers [5].

The purpose of this work was to characterize the cellular components that are part of the aggregates observed in patients with sepsis. We also used an in vitro model based on LPS stimulation to establish whether activated platelets mediate the formation of these cellular aggregates through their capacity of adhesion between themselves and to other cells of the immune system. Using a dot plot based on the size parameter (FSC-H vs FSC-A) through flow cytometry, increased number of cellular aggregates in the condition stimulated with LPS was observed. This was confirmed using Wright's stain analyzed through light field microscopy and immunofluorescence assays where the immunophenotype of cells composing the aggregates was taken into account.

The greater number of cellular aggregates found both in stimulated cells and a septic patient compared to healthy volunteers corresponds to what was previously described in systemic inflammation compared to healthy volunteers [2]. The main cells that make up these cellular aggregates are neutrophils; the interaction between them is not mediated by the platelets, however it is observed that the platelets coat the cells. This was also observed in the aggregates of the patient with sepsis. Any protein ligands or glycoproteins that bind neutrophils with other neutrophils have been reported so far, however the formation of complexes with Von Willebrand factor and tissue factor has been reported and it increases its expression in monocytes and neutrophils in processes of systemic inflammation [6]. Increased expression of ICAM-I could also favor adhesion and aggregation between neutrophils and other leukocytes such as monocytes and lymphocytes that present ligands such as LFA-1 and Mac-1 without the participation of platelets [7]. This molecule could be of research interest in the future to know the composition of cellular aggregates. However, participation of platelets cannot be ruled out since the number of experiments for the present report is small. Increased platelet-neutrophil, platelet-monocyte aggregates and microparticle formation has been observed in patients with sepsis and systemic inflammation as reported by Ogura Hiroshi [8]. Our results correlate with some aspects that are observed in sepsis, for example: neutrophilia is often reported in septic patients and it could be a contributor to the sample presenting mainly aggregates formed by this type of cells. It should be mentioned that in the in-vitro model platelets make aggregates with each other, which has been observed in patients with sepsis during endothelial failure. Besides, platelets can favor the formation of NETs and from there give rise to damage to the endothelium [9].

In conclusion, the environment given in the in-vitro model and in systemic inflammation patient's samples favors platelet activation since they form larger aggregates, however they do not function as union bridges between leukocytes (mainly neutrophils) making up cellular aggregates.

REFERENCES

[1] T. van der Poll, F.L. van de Veerdonk, B.P. Scicluna, and M.G. Netea, The immunopathology of sepsis and potential therapeutic targets. Nat Rev Immunol 17 (2017) 407-420.

[2] M.T. Gutiérrez-Ponce, Caracterización de agregados leucocitarios en pacientes con sepsis., National Autonomous University of Mexico (UNAM), Mexico City, Mexico, 2016.

[3] K. Tanaka, Y. Koike, T. Shimura, M. Okigami, S. Ide, Y. Toiyama, Y. Okugawa, Y. Inoue, T. Araki, K. Uchida, Y. Mohri, A. Mizoguchi, and M. Kusunoki, In vivo characterization of neutrophil extracellular traps in various organs of a murine sepsis model. PLoS One 9 (2014) e111888.

[4] M. Gawaz, S. Fateh-Moghadam, G. Pilz, H.J. Gurland, and K. Werdan, Platelet activation and interaction with leucocytes in patients with sepsis or multiple organ failure. Eur J Clin Invest 25 (1995) 843-51.

[5] M.T. Rondina, M. Carlisle, T. Fraughton, S.M. Brown, R.R. Miller, 3rd, E.S. Harris, A.S. Weyrich, G.A. Zimmerman, M.A. Supiano, and C.K. Grissom, Platelet-monocyte aggregate formation and mortality risk in older patients with severe sepsis and septic shock. J Gerontol A Biol Sci Med Sci 70 (2015) 225-31.

[6] R. Ehlers, V. Ustinov, Z. Chen, X. Zhang, R. Rao, F.W. Luscinskas, J. Lopez, E. Plow, and D.I. Simon, Targeting platelet-leukocyte interactions: identification of the integrin Mac-1 binding site for the platelet counter receptor glycoprotein Ibalpha. J Exp Med 198 (2003) 1077-88.

[7] A. Woodfin, M. Beyrau, M.B. Voisin, B. Ma, J.R. Whiteford, P.L. Hordijk, N. Hogg, and S. Nourshargh, ICAM-1-expressing neutrophils exhibit enhanced effector functions in murine models of endotoxemia. Blood 127 (2016) 898-907.

[8] H. Ogura, T. Kawasaki, H. Tanaka, T. Koh, R. Tanaka, Y. Ozeki, H. Hosotsubo, Y. Kuwagata, T. Shimazu, and H. Sugimoto, Activated platelets enhance microparticle formation and platelet-leukocyte interaction in severe trauma and sepsis. J Trauma 50 (2001) 801-9.

[9] B. McDonald, R.P. Davis, S.J. Kim, M. Tse, C.T. Esmon, E. Kolaczkowska, and C.N. Jenne, Platelets and neutrophil extracellular traps collaborate to promote intravascular coagulation during sepsis in mice. Blood 129 (2017) 1357-1367.

Mutations pattern of primary antibody deficiencies by next generation sequencing

Gabriela Lopez-Herrera, Laura Berron Ruiz, Alejandro Sanchez-Flores

1Instituto Nacional de Pediatria, Mexico City, Mexico
[2]National Autonomous University of Mexico, Cuernavaca, Mexico
lohegabyqbp@gmail.com; lberronruiz@yahoo.com.mx; alexsf@ibt.unam.mx

Keywords: NGS, PADs, Btk, CD40LG, LRBA

Introduction: Diagnosis of Primary immunodeficiencies (PIDs) are growing around the world, definitive diagnosis consists in determining the type of mutation presented. Diagnosis of X-linked Agammaglobulinemia (XLA), Common Variable Immunodeficiency (CVID), Severe Combined Immunodeficiency (SCID) and Hyper-IgM syndrome are the most frequent diagnosis in our Research Unit. To date, we have identified several mutations by Sanger sequencing, however, it takes a long time to detect mutations in any of the genes studied. For this reason, the main objective of this work was to employ the new generation sequencing technologies to determine the mutations presented in BTK (XLA), LRBA (CVID), IL2RG (SCID) and CD40LG (HIGM).

Objective: To determine the type of mutation associated with a cohort of patients with clinical and laboratory diagnosis of XLA, LRBA deficiency, X-SCID and X-HIGM.

Results: The design of a sequencing panel that includes BTK, LRBA, IL2RG and CD40LG was performed by using StudioDesing® online tool provided by Illumina. This panel included all the exons and the splice sites. Amplicon size was 425bp and it was obtained to sequence 96 samples. With this panel, we obtained sequencing results in 88 of 95 samples (92%) and most of the exons were covered, with the exception for exon1 for BTK, exons 1 and 2 for IL2RG and exons 43 and 49 for LRBA. With this panel, we detect 10 patients with LRBA mutations, 29 individuals with BTK deficiency and 5 and 4 genetic defects for CD40LG and IL2RG, respectively. Most of the changes were missense mutations, however, nonsense, deletions, insertions and splice site mutations were also detected.

Conclusions: We detect in total 48 individuals with the genetic deficiencies suspected. However, we still do not know the genetic deficiency in 40 of the patients included in this panel, even though they showed clinical and laboratory data to suspect of the diagnosis studied. It is interesting to perform future studies to determine if the phenotype observed are due to related genetic deficiencies already described in the literature, or to determine if there are non-described deficiencies in this cohort of patients.

Acknowledgements: FOSSIS-CONACYT 161089; CB-CONACYT 154472 and 256471.

Definite diagnosis for primary immunodeficiencies by clinical exome sequencing; a novel mutation case report

Bernard Esquivel, Karla Ruiz-Ceja and Martin Silva-Aguilar

GeneTest, Total Quality Medicine, CDMX, Mexico
bernard.esquivel@genomedik.com; karla.ruiz@genetest.com; martin.silva@pqmedicine.com

Keywords: IPEX syndrome, NGS, clinical exome sequencing, female, primary immunodeficiency, FOXP3

Background: Primary immunodeficiencies (PIDDs) are inherited disorders that occur in 1:2000 live births that affect the immune system. More than 200 distinct immune genetic disorders have been identified to date (*supp mat* Table 1) (Bonilla et al., 2015). In Mexico, PIDDs have a frequency of 1:265,000. Each year more than 4,000 children are born with PIDs where 250 will present with severe forms (Errante et al., 2012).

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome (OMIM 304790) is a rare disorder that causes life-threatening systemic autoimmunity (Gambineri et al., 2003; Torgerson et al., 2007). It is characterized by enteropathies such as severe diarrhea, chronic dermatitis, early-onset type 1 diabetes mellitus (T1DM), among others (Gambineri et al., 2008b). So far, no IPEX patients with definite mutations in the *FOXP3* gene have been reported in Mexico.

Despite the current knowledge, most PIDDs cases remain undiagnosed (Shearer et al., 2014). Next-generation sequencing (NGS) has become a valuable first-line diagnostic tool for complex genetic disorders (Chou et al., 2012). Whole Exome Sequencing (WES) analyzes 85% of genetic alterations responsible for human diseases (Bamshad et al., 2011), making it attractive for the diagnosis of patients with PIDD (Mousallem et al., 2015).

Aims: Use clinical exome sequencing (NGS) for a primary immunodeficiency diagnostic.

Methods: A female patient was diagnosed with several immune disorders. The molecular analysis was performed by DNA sequencing using high-throughput Clinical Exome Sequencing (CES) (Illumina NextSeq®) after informed consent in conformity with eth-

ical and human principles of research. Genomic DNA was obtained from buccal swabs (Oracollect®, Canada) and was amplified using PCR followed by DNA sequencing with an average depth over 100X and coverage of 99.5%. Bioinformatics analysis was performed using Sophia DDM® software 4.5.3.1 and the reference genome GRCh37/hg19. The Exome Aggregation Consortium (ExAC) (Lek et al., 2016), and G1000 (Auton et al., 2015) were used to analyze the variants frequency in the population.

Results

Patient

A 23 years old female patient from Mexican non-consanguineous parents was diagnosed with a T cell immunodeficiency. Starting at 3 months old with intolerance to breastmilk followed by severe diarrhea with poor treatment response. At 18 months old, she developed two otitis media, and at the age of 3 years old, she was admitted to the ER with severe ketoacidosis and discharged with the diagnosed of T1DM. At 20 years old, she developed maxillary abscesses treated by wide spectrum antibiotics, steroids and surgical drained reporting histopathological findings compatible with desmoid-type mandibular fibromatosis (*supp mat* Figure 1). Two years later, she developed chronic severe eczema with poor treatment response. Immunological findings showed neutrophils 84.0%, lymphocytes 14.0%, band neutrophils 13% (*supp mat* Table 2 and 3).

DNA sequence

Through CES, we found a "*de novo*" substitution of c.438C>T in exon 5 at the *FOXP3* gene (NM_001114377; rs2232367), leading to the p.Ser146Ser synonymous variant. The patient is heterozygous for this variant. ExAC and G1000 showed that this variant has a low allele frequency in population (0.032 and 0.0148 respectively).

Discussion: We report the p.S146S synonymous variant in a female patient with IPEX-like syndrome. Recessive X-linked immunodeficiencies are characterized by severe disorders in the affected males, while female carriers have a normal immunological phenotype (Tommasini et al., 2002). However, in IPEX, the enteropathy has a variable age of onset and females can be affected (Bennet et al., 2001; Barzaghi et al., 2012; Horiuchi et al., 2012; Owen et al., 2003; Zuber et al., 2007). IPEX is rare, as fewer than 150 affected worldwide with a frequency of 1 in 1.6 million people (Hannibal and Torgerson, 2011).

To date, most cases of IPEX have been associated with mutations in *FOXP3*. Many of these are missense mutations in the highly conserved winged helix domain of Foxp3, altering the DNA binding of this motif (Wildin et al., 2001) or frameshift mutations in other parts of the gene that affect the gene product (Bennet et al., 2001; Owen et al., 2003) and interfere the winged helix (Wildin et al., 2002).

Gambineri et al., (2008a) reported a silent mutation in *FOXP3* close to a splice-site at the intron 4/exon 5 boundaries that have been found in other IPEX patients. Pacheco-González et al., (2016) found an association between *FOXP3* polymorphisms and the development of autoimmune disorders. They identified the c.4572G>A (exon 8), the c.7093G>A (exon 11) and c.1651C>T (exon 5) synonymous SNPs in *FOXP3*.

Synonymous variants do not change the protein sequence, but they could be involved in transcriptional regulation affecting *FOXP3* isoforms. (Czech et al., 2010). Silent mutations can change mRNA structure, altering translation and protein folding (Saunders and Deane, 2010).

FOXP3 gene is responsible for the development of several immunological diseases (Wildin et al., 2002). As the role of *FOXP3* in regulatory T cells is to control the development of autoimmune diseases, these silent variants could participate in regulatory mechanisms implicated in these diseases.

Conclusions: We found the p.S146S synonymous variant in *FOXP3* at exon 5 in a patient with an IPEX-like syndrome. Synonymous variants could affect the expression levels of *FOXP3*, modifying its function. Immunodysregulation, polyendocrinopathy, enteropathy syndromes may be heterogeneous. Further studies are needed to fully elucidate the molecular pathogenesis and to analyze other regulatory sequences and functional analysis in *FOXP3*.

The use of sequencing technologies, such as WES, has helped to identify *de novo* mutations in the genome. This provides information on the mechanisms involved in mutagenesis and the distribution of the mutations in the genome. However, even though NGS has been used extensively in PIDD research, it has been an emerging tool in clinical immunology. WES represents a cost-effective and rapid first-line genetic approach for the evaluation of complex PIDDs as genotype–phenotype can be correlated facilitating and improving the development of further treatments. The genetic basis of PIDDs and its focus on molecular mechanisms allow patients with immune diseases ideal candidates for NGS. By knowing the diagnosis through genetic testing, we can understand more and personalize treatments for a better response.

REFERENCES

1. Al-Herz, W., Bousfiha, A., Casanova, J., Chatila, T., Conley, M., Cunningham-Rundles, C., Etzioni, A., Franco, J., Gaspar, H., Holland, S., Klein, C., Nonoyama, S., Ochs, H., Oksenhendler, E., Picard, C., Puck, J., Sullivan, K. and Tang, M. (2014). Primary Immunodeficiency Diseases: An Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. Frontiers in Immunology, 5.
2. Allan, S. E., Alstad, A. N., Merindol, N., Crellin, N. K., Amendola, M., Bacchetta, R., Naldini, L., Roncarolo, M. G., Soudeyns, H., and Levings, M. K. (2008). Generation of potent and stable human CD4+ T regulatory cells by activation-independent expression of FOXP3. Mol. Ther. 16, 194–202.
3. Allan, S. E., Crome, S. Q., Crellin, N. K., Passerini, L., Steiner, T. S., Bacchetta, R., Roncarolo, M. G., and Levings, M. K. (2007). Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. Int. Immunol. 19, 345–354.
4. Allan, S. E., Passerini, L., Bacchetta, R., Crellin, N., Dai, M., Orban, P. C., Ziegler, S. F., Roncarolo, M. G., and Levings, M. K. (2005). The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. J. Clin. Invest. 115, 3276–3284.
5. An, Y. F., Xu, F., Wang, M., Zhang, Z. Y., and Zhao, X. D. (2011). Clinical and molecular characteristics of immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome in China. Scand. J. Immunol. 74, 304–309.
6. Auton, A., Abecasis, G., Altshuler, D., Durbin, R., Abecasis, G., Bentley, D., Chakravarti, A., Clark, A., Donnelly, P., Eichler, E., Flicek, P., Gabriel, S., Gibbs, R., Green, E., Hurles, M., Knoppers, B., Korbel, J., Lander, E., Lee, C., Lehrach, H., Mardis, E., Marth, G., McVean, G., Nickerson, D., Schmidt, J., Sherry, S., Wang, J., Wilson, R., Gibbs, R., Boerwinkle, E., Doddapaneni, H., Han, Y., Korchina, V., Kovar, C., Lee, S., Muzny, D., Reid, J., Zhu, Y., Wang, J., Chang, Y., Feng, Q., Fang, X., Guo, X., Jian, M., Jiang, H., Jin, X., Lan, T., Li, G., Li, J., Li, Y., Liu, S., Liu, X., Lu, Y., Ma, X., Tang, M., Wang, B., Wang, G., Wu, H., Wu, R., Xu, X., Yin, Y., Zhang, D., Zhang, W., Zhao, J., Zhao, M., Zheng, X., Lander, E., Altshuler, D., Gabriel, S., Gupta, N., Gharani, N., Toji, L., Gerry, N., Resch, A., Flicek, P., Barker, J., Clarke, L., Gil, L., Hunt, S., Kelman, G., Kulesha, E., Leinonen, R., McLaren, W., Radhakrishnan, R., Roa, A., Smirnov, D., Smith, R., Streeter, I., Thormann, A., Toneva, I., Vaughan, B., Zheng-Bradley, X., Bentley, D., Grocock, R., Humphray, S., James, T., Kingsbury, Z., Lehrach, H., Sudbrak, R., Albrecht, M., Amstislavskiy, V., Borodina, T., Lienhard, M., Mertes, F., Sultan, M., Timmermann, B., Yaspo, M., Mardis, E., Wilson, R., Fulton, L., Fulton, R., Sherry, S., Ananiev, V., Belaia, Z., Beloslyudtsev, D., Bouk, N., Chen, C., Church, D., Cohen, R., Cook, C., Garner, J., Hefferon, T., Kimelman, M., Liu, C., Lopez, J., Meric, P., O'Sullivan, C., Ostapchuk, Y., Phan, L., Ponomarov, S., Schneider, V., Shekhtman, E., Sirotkin, K., Slotta, D., Zhang, H., McVean, G., Durbin, R., Balasubramaniam, S., Burton, J., Danecek, P., Keane, T., Kolb-Kokocinski, A., McCarthy, S., Stalker, J., Quail, M., Schmidt, J., Davies, C., Gollub, J., Webster, T., Wong, B., Zhan, Y., Auton, A., Campbell, C., Kong, Y., Marcketta, A., Gibbs, R., Yu, F., Antunes, L., Bainbridge, M., Muzny, D., Sabo, A., Huang, Z., Wang, J., Coin, L., Fang, L., Guo, X., Jin, X., Li, G., Li, Q., Li, Y., Li, Z., Lin, H., Liu, B., Luo, R., Shao, H., Xie, Y., Ye, C., Yu, C., Zhang, F., Zheng, H., Zhu, H., Alkan, C., Dal, E., Kahveci, F., Marth, G., Garrison, E., Kural, D., Lee, W., Fung Leong, W., Stromberg, M., Ward, A., Wu, J., Zhang, M., Daly, M., DePristo, M., Handsaker, R., Altshuler, D., Banks, E., Bhatia, G., del Angel, G., Gabriel, S., Genovese, G., Gupta, N., Li, H., Kashin, S., Lander, E., McCarroll, S., Nemesh, J., Poplin, R., Yoon, S., Lihm, J., Makarov, V., Clark, A., Gottipati, S., Keinan, A., Rodriguez-Flores, J., Korbel, J., Rausch, T., Fritz, M., Stütz, A., Flicek, P., Beal, K., Clarke, L., Datta, A., Herrero, J., McLaren, W., Ritchie, G., Smith, R., Zerbino, D., Zheng-Bradley, X., Sabeti, P., Shlyakhter, I., Schaffner, S., Vitti, J., Cooper, D., Ball, E., Stenson, P., Bentley, D., Barnes, B., Bauer, M., Keira Cheetham, R., Cox, A., Eberle, M., Humphray, S., Kahn, S., Murray, L., Peden, J., Shaw, R., Kenny, E., Batzer, M., Konkel, M., Walker, J., MacArthur, D., Lek, M., Sudbrak, R., Amstislavskiy, V., Herwig, R., Mardis, E., Ding, L., Koboldt, D., Larson, D., Ye, K., Gravel, S., Swaroop, A., Chew, E., Lappalainen, T., Erlich, Y., Gymrek, M., Frederick Willems, T., Simpson, J., Shriver, M., Rosenfeld, J.,

Bustamante, C., Montgomery, S., De La Vega, F., Byrnes, J., Carroll, A., DeGorter, M., Lacroute, P., Maples, B., Martin, A., Moreno-Estrada, A., Shringarpure, S., Zakharia, F., Halperin, E., Baran, Y., Lee, C., Cerveira, E., Hwang, J., Malhotra, A., Plewczynski, D., Radew, K., Romanovitch, M., Zhang, C., Hyland, F., Craig, D., Christoforides, A., Homer, N., Izatt, T., Kurdoglu, A., Sinari, S., Squire, K., Sherry, S., Xiao, C., Sebat, J., Antaki, D., Gujral, M., Noor, A., Ye, K., Burchard, E., Hernandez, R., Gignoux, C., Haussler, D., Katzman, S., James Kent, W., Howie, B., Ruiz-Linares, A., Dermitzakis, E., Devine, S., Abecasis, G., Min Kang, H., Kidd, J., Blackwell, T., Caron, S., Chen, W., Emery, S., Fritsche, L., Fuchsberger, C., Jun, G., Li, B., Lyons, R., Scheller, C., Sidore, C., Song, S., Sliwerska, E., Taliun, D., Tan, A., Welch, R., Kate Wing, M., Zhan, X., Awadalla, P., Hodgkinson, A., Li, Y., Shi, X., Quitadamo, A., Lunter, G., McVean, G., Marchini, J., Myers, S., Churchhouse, C., Delaneau, O., Gupta-Hinch, A., Kretzschmar, W., Iqbal, Z., Mathieson, I., Menelaou, A., Rimmer, A., Xifara, D., Oleksyk, T., Fu, Y., Liu, X., Xiong, M., Jorde, L., Witherspoon, D., Xing, J., Eichler, E., Browning, B., Browning, S., Hormozdiari, F., Sudmant, P., Khurana, E., Durbin, R., Hurles, M., Tyler-Smith, C., Albers, C., Ayub, Q., Balasubramaniam, S., Chen, Y., Colonna, V., Danecek, P., Jostins, L., Keane, T., McCarthy, S., Walter, K., Xue, Y., Gerstein, M., Abyzov, A., Balasubramanian, S., Chen, J., Clarke, D., Fu, Y., Harmanci, A., Jin, M., Lee, D., Liu, J., Jasmine Mu, X., Zhang, J., Zhang, Y., Li, Y., Luo, R., Zhu, H., Alkan, C., Dal, E., Kahveci, F., Marth, G., Garrison, E., Kural, D., Lee, W., Ward, A., Wu, J., Zhang, M., McCarroll, S., Handsaker, R., Altshuler, D., Banks, E., del Angel, G., Genovese, G., Hartl, C., Li, H., Kashin, S., Nemesh, J., Shakir, K., Yoon, S., Lihm, J., Makarov, V., Degenhardt, J., Korbel, J., Fritz, M., Meiers, S., Raeder, B., Rausch, T., Stütz, A., Flicek, P., Paolo Casale, F., Clarke, L., Smith, R., Stegle, O., Zheng-Bradley, X., Bentley, D., Barnes, B., Keira Cheetham, R., Eberle, M., Humphray, S., Kahn, S., Murray, L., Shaw, R., Lameijer, E., Batzer, M., Konkel, M., Walker, J., Ding, L., Hall, I., Ye, K., Lacroute, P., Lee, C., Cerveira, E., Malhotra, A., Hwang, J., Plewczynski, D., Radew, K., Romanovitch, M., Zhang, C., Craig, D., Homer, N., Church, D., Xiao, C., Sebat, J., Antaki, D., Bafna, V., Michaelson, J., Ye, K., Devine, S., Gardner, E., Abecasis, G., Kidd, J., Mills, R., Dayama, G., Emery, S., Jun, G., Shi, X., Quitadamo, A., Lunter, G., McVean, G., Chen, K., Fan, X., Chong, Z., Chen, T., Witherspoon, D., Xing, J., Eichler, E., Chaisson, M., Hormozdiari, F., Huddleston, J., Malig, M., Nelson, B., Sudmant, P., Parrish, N., Khurana, E., Hurles, M., Blackburne, B., Lindsay, S., Ning, Z., Walter, K., Zhang, Y., Gerstein, M., Abyzov, A., Chen, J., Clarke, D., Lam, H., Jasmine Mu, X., Sisu, C., Zhang, J., Zhang, Y., Gibbs, R., Yu, F., Bainbridge, M., Challis, D., Evani, U., Kovar, C., Lu, J., Muzny, D., Nagaswamy, U., Reid, J., Sabo, A., Yu, J., Guo, X., Li, W., Li, Y., Wu, R., Marth, G., Garrison, E., Fung Leong, W., Ward, A., del Angel, G., DePristo, M., Gabriel, S., Gupta, N., Hartl, C., Poplin, R., Clark, A., Rodriguez-Flores, J., Flicek, P., Clarke, L., Smith, R., Zheng-Bradley, X., MacArthur, D., Mardis, E., Fulton, R., Koboldt, D., Gravel, S., Bustamante, C., Craig, D., Christoforides, A., Homer, N., Izatt, T., Sherry, S., Xiao, C., Dermitzakis, E., Abecasis, G., Min Kang, H., McVean, G., Gerstein, M., Balasubramanian, S., Habegger, L., Yu, H., Flicek, P., Clarke, L., Cunningham, F., Dunham, I., Zerbino, D., Zheng-Bradley, X., Lage, K., Berg Jespersen, J., Horn, H., Montgomery, S., DeGorter, M., Khurana, E., Tyler-Smith, C., Chen, Y., Colonna, V., Xue, Y., Gerstein, M., Balasubramanian, S., Fu, Y., Kim, D., Auton, A., Marcketta, A., Desalle, R., Narechania, A., Wilson Sayres, M., Garrison, E., Handsaker, R., Kashin, S., McCarroll, S., Rodriguez-Flores, J., Flicek, P., Clarke, L., Zheng-Bradley, X., Erlich, Y., Gymrek, M., Frederick Willems, T., Bustamante, C., Mendez, F., David Poznik, G., Underhill, P., Lee, C., Cerveira, E., Malhotra, A., Romanovitch, M., Zhang, C., Abecasis, G., Coin, L., Shao, H., Mittelman, D., Tyler-Smith, C., Ayub, Q., Banerjee, R., Cerezo, M., Chen, Y., Fitzgerald, T., Louzada, S., Massaia, A., McCarthy, S., Ritchie, G., Xue, Y., Yang, F., Gibbs, R., Kovar, C., Kalra, D., Hale, W., Muzny, D., Reid, J., Wang, J., Dan, X., Guo, X., Li, G., Li, Y., Ye, C., Zheng, X., Altshuler, D., Flicek, P., Clarke, L., Zheng-Bradley, X., Bentley, D., Cox, A., Humphray, S., Kahn, S., Sudbrak, R., Albrecht, M., Lienhard, M., Larson, D., Craig, D., Izatt, T., Kurdoglu, A., Sherry, S., Xiao, C., Haussler, D., Abecasis, G., McVean, G., Durbin, R., Balasubramaniam, S., Keane, T., McCarthy, S., Stalker, J., Chakravarti, A., Knoppers, B., Abecasis, G., Barnes, K., Beiswanger, C., Burchard, E., Bustamante, C., Cai, H., Cao, H., Durbin, R., Gerry, N., Gharani, N., Gibbs, R., Gignoux, C., Gravel, S., Henn, B., Jones, D., Jorde, L., Kaye, J., Keinan, A., Kent, A., Kerasidou, A., Li, Y., Mathias, R., McVean, G., Moreno-Estrada, A., Ossorio, P., Parker, M., Resch, A., Rotimi, C., Royal, C., Sandoval, K., Su, Y., Sudbrak, R., Tian, Z., Tishkoff, S., Toji, L., Tyler-Smith, C., Via, M., Wang, Y., Yang, H., Yang, L., Zhu, J., Bodmer, W.,

Bedoya, G., Ruiz-Linares, A., Cai, Z., Gao, Y., Chu, J., Peltonen, L., Garcia-Montero, A., Orfao, A., Dutil, J., Martinez-Cruzado, J., Oleksyk, T., Barnes, K., Mathias, R., Hennis, A., Watson, H., McKenzie, C., Qadri, F., LaRocque, R., Sabeti, P., Zhu, J., Deng, X., Sabeti, P., Asogun, D., Folarin, O., Happi, C., Omoniwa, O., Stremlau, M., Tariyal, R., Jallow, M., Sisay Joof, F., Corrah, T., Rockett, K., Kwiatkowski, D., Kooner, J., Tịnh Hiê`n, T., Dunstan, S., Thuy Hang, N., Fonnie, R., Garry, R., Kanneh, L., Moses, L., Sabeti, P., Schieffelin, J., Grant, D., Gallo, C., Poletti, G., Saleheen, D., Rasheed, A., Brooks, L., Felsenfeld, A., McEwen, J., Vaydylevich, Y., Green, E., Duncanson, A., Dunn, M., Schloss, J., Wang, J., Yang, H., Auton, A., Brooks, L., Durbin, R., Garrison, E., Min Kang, H., Korbel, J., Marchini, J., McCarthy, S., McVean, G. and Abecasis, G. (2015). A global reference for human genetic variation. Nature, 526(7571), pp.68-74.

7. Bacchetta, R., Passerini, L., Gambineri, E., Dai, M., Allan, S. E., Perroni, L., Dagna-Bricarelli, F., Sartirana, C., Matthes-Martin, S., Lawitschka, A., Azzari, C., Ziegler, S. F., Levings, M. K., and Roncarolo, M. G. (2006). Defective regulatory and effector T cell functions in patients with FOXP3 mutations. J. Clin. Invest. 116, 1713–1722.
8. Bae, K. W., Kim, B. E., Choi, J. H., Lee, J. H., Park, Y. S., Kim, G. H., Yoo, H. W., and Seo, J. J. (2011). A novel mutation and unusual clinical features in a patient with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Eur. J. Pediatr. 170, 1611–1615
9. Bamshad M. J., Ng S. B., Bigham A. W., Tabor H. K., Emond M. J., Nickerson D. A., et al. Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet (2011) 12:745–55.
10. Baron, U., Floess, S., Wieczorek, G., Baumann, K., Grutzkau, A., Dong, J., Thiel, A., Boeld, T. J., Hoffmann, P., Edinger, M., Turbachova, I., Hamann, A., Olek, S., and Huehn, J. (2007). DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. Eur. J. Immunol. 37, 2378–2389.
11. Barzaghi, F., Passerini, L., Gambineri, E., Ciullini Mannurita, S., Cornu, T., Kang, E., Choe, Y., Cancrini, C., Corrente, S., Ciccocioppo, R., Cecconi, M., Zuin, G., Discepolo, V., Sartirana, C., Schmidtko, J., Ikinciogullari, A., Ambrosi, A., Roncarolo, M., Olek, S. and Bacchetta, R. (2012). Demethylation analysis of the FOXP3 locus shows quantitative defects of regulatory T cells in IPEX-like syndrome. Journal of Autoimmunity, 38(1), pp.49-58.
12. Barzaghi, F., Passerini, L., Gambineri, E., Ciullini Mannurita, S., Cornu, T., Kang, E. S., Choe, Y. H., Cancrini, C., Corrente, S., Ciccocioppo, R., Cecconi, M., Zuin, G., Discepolo, V., Sartirana, C., Schmidtko, J., Ikinciogullari, A., Ambrosi, A., Roncarolo, M. G., Olek, S., and Bacchetta, R. (2012). Demethylation analysis of the FOXP3 locus shows quantitative defects of regulatory T cells in IPEX-like syndrome. J. Autoimmun. 38, 49–58.
13. Battaglia, M., Stabilini, A., Migliavacca, B., Horejs-Hoeck, J., Kaupper, T., and Roncarolo, M. G. (2006). Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. J. Immunol. 177, 8338–8347.
14. Baud, O., Goulet, O., Canioni, D., Le Deist, F., Radford, I., Rieu, D., Dupuis-Girod, S., Cerf-Bensussan, N., Cavazzana-Calvo, M., Brousse, N., Fischer, A., and Casanova, J. L. (2001). Treatment of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) by allogeneic bone marrow transplantation. N. Engl. J. Med. 344, 1758–1762.
15. Bennett C. L., Christie J., Ramsdell F., Brunkow M. E., Ferguson P. J., Whitesell L., Kelly T. E., Saulsbury F. T., Chance P. F., Ochs H. D. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001; 27:20–1.
16. Bennett, C. L., Brunkow, M. E., Ramsdell, F., O'Briant, K. C., Zhu, Q., Fuleihan, R. L., Shigeoka, A. O., Ochs, H. D., and Chance, P. F. (2001a). A rare polyadenylation signal mutation of the FOXP3 gene (AAUAAA → AAUGAA) leads to the IPEX syndrome. Immunogenetics 53, 435–439.

17. Bennett, C. L., Christie, J., Ramsdell, F., Brunkow, M. E., Ferguson, P. J., Whitesell, L., Kelly, T. E., Saulsbury, F. T., Chance, P. F., and Ochs, H. D. (2001b). The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat. Genet. 27, 20–21

18. Bennett, C., Brunkow, M., Ramsdell, F., O'Briant, K., Zhu, Q., Fuleihan, R., Shigeoka, A., Ochs, H. and Chance, P. (2001). A rare polyadenylation signal mutation of the FOXP3 gene (AAUAAA→AAUGAA) leads to the IPEX syndrome. Immunogenetics, 53(6), pp.435-439.

19. Bennett, C.L., Christie, J., Ramsdell, F., Brunkow, M.E., Ferguson, P.J., Whitesell, L. et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001; 27:20–21

20. Chatila T. A., Blaeser F., Ho N., Lederman H. M., Voulgaropoulos C., Helms C., Bowcock A. M. (2000). JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. J Clin Invest 106:R75–81.

21. Chou J., Ohsumi T. K., Geha R. S. Use of whole exome and genome sequencing in the identification of genetic causes of primary immunodeficiency. Curr Opin. Allergy Clin. Immunol. (2012) 12:623–8.

22. Czech A., Fedyunin I., Zhang G., Ignatova Z. (2010). Silent mutations insight: co-variations in tRNA abundance as a key to unravel con sequences of silent mutations. Mol. Biosyst. 6:1767–72.

23. D'Hennezel, E., Ben-Shoshan, M., Ochs, H. D., Torgerson, T. R., Russell, L. J., Lejtenyi, C., Noya, F. J., Jabado, N., Mazer, B., and Piccirillo, C. A. (2009). FOXP3 forkhead domain mutation and regulatory T cells in the IPEX syndrome. N. Engl. J. Med. 361, 1710–1713.

24. De Benedetti, F., Insalaco, A., Diamanti, A., Cortis, E., Muratori, F., Lamioni, A., Carsetti, R., Cusano, R., De Vito, R., Perroni, L., Gambarara, M., Castro, M., Bottazzo, G. F., and Ugazio, A. G. (2006). Mechanistic associations of a mild phenotype of immunodysregulation, polyendocrinopathy, enteropathy, x-linked syndrome. Clin. Gastroenterol. Hepatol. 4, 653–659.

25. Di Nunzio, S., Cecconi, M., Passerini, L., Mcmurchy, A. N., Baron, U., Turbachova, I., Vignola, S., Valencic, E., Tommasini, A., Junker, A., Cazzola, G., Olek, S., Levings, M. K., Perroni, L., Roncarolo, M. G., and Bacchetta, R. (2009). Wild-type FOXP3 is selectively active in CD4+CD25(hi) regulatory T cells of healthy female carriers of different FOXP3 mutations. Blood 114, 4138–4141

26. Dorsey, M. J., Petrovic, A., Morrow, M. R., Dishaw, L. J., and Sleasman, J. W. (2009). FOXP3 expression following bone marrow transplantation for IPEX syndrome after reduced-intensity conditioning. Immunol. Res. 44, 179–184.

27. Dvorak C. C., Cowan M. J., Logan B. R., Notarangelo L. D., Griffith L. M., Puck J. M., et al. The natural history of children with severe combined immunodeficiency: baseline features of the first fifty patients of the Primary Immune Deficiency Treatment Consortium prospective study 6901. J Clin. Immunol. (2013) 33:1156–64

28. Edghill, E. L., Flanagan, S. E., Patch, A. M., Boustred, C., Parrish, A., Shields, B., Shepherd, M. H., Hussain, K., Kapoor, R. R., Malecki, M., Macdonald, M. J., Stoy, J., Steiner, D. F., Philipson, L. H., Bell, G. I., Hattersley, A. T., and Ellard, S. (2008). Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. Diabetes 57, 1034–1042.

29. Errante, P., Franco, J., Espinosa-Rosales, F., Sorensen, R. and Condino-Neto, A. (2012). Advances in primary immunodeficiency diseases in Latin America: epidemiology, research, and perspectives. Annals of the New York Academy of Sciences, 1250(1), pp.62-72.

30. Ferguson, P. J., Blanton, S. H., Saulsbury, F. T., Mcduffie, M. J., Lemahieu, V., Gastier, J. M., Francke, U., Borowitz, S. M., Sutphen, J. L., and Kelly, T. E. (2000). Manifestations and linkage analysis in X-linked autoimmunity-immunodeficiency syndrome. Am. J. Med. Genet. 90, 390–397.

31. Fontenot, J. D., Gavin, M. A., and Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat. Immunol. 4, 330–336.

32. Fuchizawa, T., Adachi, Y., Ito, Y., Higashiyama, H., Kanegane, H., Futatani, T., Kobayashi, I., Kamachi, Y., Sakamoto, T., Tsuge, I., Tanaka, H., Banham, A. H., Ochs, H. D., and Miyawaki, T. (2007). Developmental changes of FOXP3-expressing CD4+CD25+ regulatory T cells and their impairment in patients with FOXP3 gene mutations. Clin. Immunol. 125, 237–246.

33. Gallo, V., Dotta, L., Giardino, G., Cirillo, E., Lougaris, V., D'Assante, R., Prandini, A., Consolini, R., Farrow, E., Thiffault, I., Saunders, C., Leonardi, A., Plebani, A., Badolato, R. and Pignata, C. (2016). Diagnostics of Primary Immunodeficiencies through Next-Generation Sequencing. Frontiers in Immunology, 7.

34. Gambineri E., Torgerson T. R., Ochs H. D. (2003). Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. Curr Opin Rheumatol. 15:430–5.

35. Gambineri, E., Bianchi, L., Mannurita, S., Gelli, A., Lionetti, P., Martino, M. and Azzari, C. (2008a). F.80. A Silent FOXP3 Mutation Possibly Responsible of Atypical IPEX (Immune- Dysregulation, Polyendocrinopathy, Enteropathy, X-linked) Phenotype. Clinical Immunology, 127, p.S69.

36. Gambineri, E., Perroni, L., Passerini, L., Bianchi, L., Doglioni, C., Meschi, F., Bonfanti, R., Sznajer, Y., Tommasini, A., Lawitschka, A., Junker, A., Dunstheimer, D., Heidemann, P., Cazzola, G., Cipolli, M., Friedrich, W., Janic, D., Azzi, N., Richmond, E., Vignola, S., Barabino, A., Chiumello, G., Azzari, C., Roncarolo, M. and Bacchetta, R. (2008b). Clinical and molecular profile of a new series of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: Inconsistent correlation between forkhead box protein 3 expression and disease severity. Journal of Allergy and Clinical Immunology, 122(6), pp.1105-1112.e1.

37. Gambineri, E., Perroni, L., Passerini, L., Bianchi, L., Doglioni, C., Meschi, F., Bonfanti, R., Sznajer, Y., Tommasini, A., Lawitschka, A., Junker, A., Dunstheimer, D., Heidemann, P. H., Cazzola, G., Cipolli, M., Friedrich, W., Janic, D., Azzi, N., Richmond, E., Vignola, S., Barabino, A., Chiumello, G., Azzari, C., Roncarolo, M. G., and Bacchetta, R. (2008). Clinical and molecular profile of a new series of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: inconsistent correlation between forkhead box protein 3 expression and disease severity. J. Allergy Clin. Immunol.122, 1105–1112.e1101

38. Gambineri, E., Torgerson, T. R., and Ochs, H. D. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. Curr Opin Rheumatol. 2003; 15:430–435.

39. Geha, R. S., L. D., Notarangelo, J.-L Casanova, et al. (2007). Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. J. Allergy Clin. Immunol. 120:776–794.

40. Hannibal M. C., Torgerson T., IPEX Syndrome. 2004 Oct 19 [Updated 2011 Jan 27]. In: Adam M. P., Ardinger H. H., Pagon R. A., et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1118/.

41. Horiuchi, S., Ishiguro, A., Nakagawa, T., Shoji, K., Nagai, A., Arai, K., Horikawa, R., Kawai, T., Watanabe, N. and Onodera, M. (2012). A girl of IPEX syndrome with low expression of FOXP3. Japanese Journal of Clinical Immunology, 35(6), pp.526-532.

42. Lek, M., Karczewski, K. J., Minikel, E. V., Samocha, K. E., Banks, E., Fennell, T., O'Donnell-Luria, A. H., Ware, J. S., Hill, A. J., Cummings, B. B., Tukiainen, T., Birnbaum, D. P., Kosmicki, J. A., Duncan, L. E., Estrada, K., Zhao, F., Zou, J., Pierce-Hoffman, E., Berghout, J., Cooper, D. N., Deflaux, N., DePristo, M., Do, R., Flannick, J., Fromer, M., Gauthier, L., Goldstein, J., Gupta, N., Howrigan, D., Kiezun, A., Kurki, M. I., Moonshine, A. L., Natarajan, P., Orozco, L., Peloso, G. M., Poplin, R., Rivas, M. A., Ruano-Rubio, V., Rose, S. A., Ruderfer, D. M., Shakir, K., Stenson, P. D., Stevens, C., Thomas, B. P., Tiao, G., Tusie-Luna, M. T., Weisburd, B., Won, H. H., Yu, D., Altshuler, D. M., Ardissino, D., Boehnke, M., Danesh, J., Donnelly, S., Elosua, R., Florez, J. C.,

Gabriel, S. B., Getz, G., Glatt, S. J., Hultman, C. M., Kathiresan, S., Laakso, M., McCarroll, S., McCarthy, M. I., McGovern, D., McPherson, R., Neale, B. M., Palotie, A., Purcell, S. M., Saleheen, D., Scharf, J. M., Sklar, P., Sullivan, P. F., Tuomilehto, J., Tsuang, M. T., Watkins, H. C., Wilson, J. G., Daly, M. J. & MacArthur, D. G. (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536(7616), 285–91.

43. Mousallem T., Urban T. J., McSweeney K. M., Kleinstein S. E., Zhu M., Adeli M., et al. Clinical application of whole-genome sequencing in patients with primary immunodeficiency. J Allergy Clin. Immunol. (2015) 136:476–9.e6.

44. Ochs, H. and Hagin, D. (2014). Primary immunodeficiency disorders: general classification, new molecular insights, and practical approach to diagnosis and treatment. Annals of Allergy, Asthma & Immunology, 112(6), pp.489-495.

45. Owen, C., Jennings, C., Imrie, H., Lachaux, A., Bridges, N., Cheetham, T. and Pearce, S. (2003). Mutational Analysis of the FOXP3 Gene and Evidence for Genetic Heterogeneity in the Immunodysregulation, Polyendocrinopathy, Enteropathy Syndrome. The Journal of Clinical Endocrinology & Metabolism, 88(12), pp.6034-6039.

46. Pacheco-Gonzalez, R., Avila, C., Dávila, I., García-Sánchez, A., Hernández-Hernández, L., Benito-Pescador, D., Torres, R., Prieto-Matos, P., Isidoro-Garcia, M., Lorente, F. and Sanz, C. (2016). Analysis of FOXP3 gene in children with allergy and autoimmune diseases. Allergologia et Immunopathologia, 44(1), pp.32-40.

47. Saunders R., and Deane C. M. (2010). Synonymous codon usage influences the local protein structure observed. Nucleic Acids Res. 38:6719–28.

48. Shearer W. T., Dunn E., Notarangelo L. D., Dvorak C. C., Puck J. M., Logan B. R., et al. Establishing diagnostic criteria for severe combined immunodeficiency disease (SCID), leaky SCID, and Omenn syndrome: the primary immune deficiency treatment consortium experience. J Allergy Clin. Immunol. (2014) 133:1092–8.

49. Tommasini, A., Ferrari, S., Moratto, D., Badolato, R., Boniotto, M., Pirulli, D., Notarangelo, L. and andolina, M. (2002). X-chromosome inactivation analysis in a female carrier of FOXP3 mutation. Clinical and Experimental Immunology, 130(1), pp.127-130.

50. Torgerson T. R., Linane A., Moes N., Anover S., Mateo V., Rieux-Laucat F., Hermine O., Vijay S., Gambineri E., Cerf-Bensussan N., Fischer A., Ochs H. D., Goulet O., Ruemmele F. M. (2007). Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene. Gastroenterology; tor132:1705–17.

51. Verbsky, J. W., & Chatila, T. A. (2013). Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) and IPEX-Related Disorders: an Evolving Web of Heritable Autoimmune Diseases. Current Opinion in Pediatrics, 25(6), 708–714. http://doi.org/10.1097/MOP.0000000000000029

52. Wildin R. S., Smyk-Pearson S., Filipovich A. H. (2002). Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet. 39:537–45.

53. Wildin, R. S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J. L., Buist, N. et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet. 2001; 27:18–20.

54. Zuber, J., Viguier, M., Lemaitre, F., Senée, V., Patey, N., Elain, G., Geissmann, F., Fakhouri, F., Ferradini, L., Julier, C. and Bandeira, A. (2007). Severe FOXP3+ and Naïve T Lymphopenia in a Non-IPEX Form of Autoimmune Enteropathy Combined With an Immunodeficiency. Gastroenterology, 132(5), pp.1694-1704.

Characterization and evolution of peripheral lymphocyte populations in patients affected by Alzheimer and Parkinson disease

Andrea Alejandra Toledo[1], Beatriz Tamaya[2], Santiago Garfias[2], Mariana Arroyo[2], Ulises Rodriguez[2], Catherine Boll[2], Ana Luisa Sosa[2], Edda Sciutto[3], Gladis Fragoso[3], Agnes Fleury[2,3]

[1]Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[2]Instituto Nacional de Neurología y Neurocirugía (INNN), Mexico City, Mexico
[3]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
antoro06@yahoo.com; bettytd8@gmail.com; dr.garfias@gmail.com; marroyot@gmail.com; ulrdz@hotmail.com; bollneur@gmail.com; drsosa@hotmail.com; edda@unam.mx; gladis@biomedicas.unam.mx; afleury@biomedicas.unam.mx

Keywords: Alzheimer Disease, Parkinson Disease, Inflammation., lymphocyte, Key words: Neurodegeneration

Neuroinflammation is involved in the pathophysiology of a growing number of neurological disorders, including Alzheimer disease (AD) and Parkinson disease (PD) (Prince, 2013, Pringsheim 2014). The prevalence of neurodegenerative diseases is continuously rising, mainly due to the increased human lifespan (Dorsey, 2007; Hebert 2013). While the most evident risk factor is older age, other factors are also involved (Collier, 2011; Udeochu, 2016). At a clinical level, AD is characterized by memory loss and cognitive deterioration on the other hand, although non-motor symptoms do exist in PD, the hallmarks of the disease are tremor, bradykinesia, and rigid muscles (Poewe, 2017). At a pathological level, both disorders are characterized by a progressive neuronal loss, principally in the cerebral cortex, hippocampus, and amygdala in AD, and mostly restricted to dopaminergic neurons of the substantia nigra pars compacta in PD (Simon, 2011). It is now clear that a neuroinflammatory response is present in both diseases. Microglia and astrocyte activation, the presence of proinflammatory cytokines and alterations in the blood-brain barrier that allow peripheral cells to infiltrate the central nervous system have been evidenced in both diseases (Rocha, 2015; Mietelska-Porowska, 2017). Although the role of neuroinflammation is not completely understood, it is generally regarded as a deleterious phenomenon involved in the neurodegenerative process (Appel, 2009; Bagyinszky, 2017).

The present study was designed to further explore peripheral changes in lymphocyte subpopulations in AD and PD patients and to identify alterations that could help us to predict disease progression.

Table 1: Mean characteristics of included patients and controls

	AD (n=20)	Controls AD (n=15)	PD (n= 20)	Controls PD (n=18)
Gender (M / F)	8 / 12	6 / 9	10 / 10	7 / 11
Age (years) mean ±SD (Min-Max)	74 ± 8.0 (55-84)	69.1 ± 5.7 (61-81)	64.7 ± 9.5 (43-81)	67.5 ± 6.2 (60-81)
Months of evolution mean ±SD (Min-Max)	63.0 ± 41.4 (17-180)		123.3±68.4 (7-252)	
Scale scores at T1 mean±SD (min-max)	27.3 ± 13.4* (5-53)		2.2 ± 0.7[$] (1-3)	
Scale scores at T2 mean±SD (min-max)	30.0 ± 15.0* (9-51)		2.5 ± 0.5[$] (1-3)	

*DSRS (Dementia Severity Rating scale); [$]H & Y (Hoehn and Yahr scale).

Table 2: Antibody combinations used for FACS

Antibodies	Characteristic
CD4+ (FITC)	Total T helper cells
CD8+ (FITC)	Total cytotoxic T cells
CD4+/CD8+ CD69+ (PerCP)	Early activated
CD4+/CD8+ CD38+ (APC)	Late activated
CD4+/CD8+ CD95+ (APC)	Fas expression
CD4+/CD8+ CD45RA+ CD45RO- CCR7+ (PE, PerCP, Cy5.5, alexa 647, respectively)	Naive lymphocytes (T_N)
CD4+/CD8+ CD45RA+CD45RO-CCR7-	Effector lymphocytes (T_E)
CD4+/CD8+ CD45RA-CD45RO+CCR7-	Effector memory lymphocytes (T_{EM})
CD4+/CD8+ CD45RA-CD45RO+CCR7+	Central memory lymphocytes (T_{CM})
CD4+CD25+FoxP3+CD45RO+ (APC, PE, FITC, respectively)	Activated T regulator cells
CD19+ (FITC)	Total B cells
CD19+CD5+FoxP3 (PerCP, PE, respectively)	Activated B regulator cells
CD19+CD5+IL10+ (APC)	B cells expressing IL10
CD19+CD5+FoxP3+IL10+	Activated B regulator cells expressing IL10

FITC: fluorescein isothiocyanate; PerCP: peridinin chlorophyll; APC: allophycocyanin; PE: phycoerythrin

For this purpose we evaluate the peripheral lymphocyte profile in 20 AD patients, 20 PD patients, and a group of healthy individuals. Ten of the AD and 12 of the PD patients were studied again 17 to 27 months later (Table 1). Lymphocyte subsets and their activation status were determined by flow cytometry using different antibody combination (Table 2). All patients were neurologically evaluated using internationally validated scales.

Both AD and PD patients showed a significant increase in the levels of activated lymphocytes, apoptosis-susceptible lymphocytes, central memory T cells, and T and B cells with regulatory phenotypes with respect to healthy subjects. Also, as the disease progresses, a significant decline in activated and regulatory cells was observed. In AD, higher progression rates were associated with lower percentages of CD4+CD38+ and higher percentages of CD4TE cells at the beginning of the study. Significant differences between both diseases were observed.

This study provides evidence of changes in peripheral lymphocyte phenotypes associated to AD and PD and their severity. Considering the effective blood-brain communication, our results open new avenues to explore immunomodulation therapies to treat these diseases.

REFERENCES

Appel, S.H. (2009). CD4+ T cells mediate cytotoxicity in neurodegenerative diseases. J Clin Invest. 119:13-15. doi: 10.1172/JCI38096

Bagyinszky, E., Giau, V.V., Shim, K., Suk, K., An, S.S.A., Kim, S.(2017). Role of inflammatory molecules in the Alzheimer's disease progression and diagnosis. J Neurol Sci. 376:242-254. doi: 10.1016/j.jns.2017.03.031.

Collier, T.J., Kanaan, N.M., Kordower, J.H. (2011). Ageing as a primary risk factor for Parkinson's disease: evidence from studies of non-human primates. Nat Rev Neurosci. 12:359-366. doi: 10.1038/nrn3039

Dorsey, E.R., Constantinescu, R., Thompson, J.P., Biglan, K.M., Holloway, R.G., Kieburtz K, et al. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology. 68:384-386. doi:10.1212/01.wnl.0000247740.47667.03

Hebert, L.E., Weuve, J., Scherr, P.A., Evans, D.A. (2013). Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology. 80:1778-1783. doi: 10.1212/WNL.0b013e31828726f5

Mietelska-Porowska, A., Wojda, U. (2017). T Lymphocytes and Inflammatory Mediators in the Interplay between Brain and Blood in Alzheimer's Disease: Potential Pools of New Biomarkers. J Immunol Res. 2017:4626540. doi.org/10.1155/2017/4626540

Poewe, W., Seppi, K., Tanner, C.M., Halliday, G.M., Brundin, P., Volkmann, J., et al. (2017). Parkinson disease. Nat Rev Dis Primers. 3:17013. doi: 10.1038/nrdp.2017.13.

Prince, M., Bryce, R., Albanese, E. Wimo, A., Ribeiro, W., Ferri, C.P. (2013). The global prevalence of dementia: a systematic review and metaanalysis. Alzheimers Dement. 9:63-75.e2. doi: 10.1016/j.jalz.2012.11.007.

Pringsheim, T., Jette, N., Frolkis, A., Steeves, T.D. (2014). The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord. 29:1583-1590. doi: 10.1002/mds.25945

Rocha, N.P., de Miranda, A.S., Teixeira, A.L. (2015). Insights into Neuroinflammation in Parkinson's Disease: From Biomarkers to Anti-Inflammatory Based Therapies. Biomed Res Int. 2015:628192. doi: 10.1155/2015/628192

Simon Lunn, J., Sakowski, S.A., Hur, J., Feldman, e.L. (2011). Stem Cell Technology for Neurodegenerative Diseases. Ann Neurol 2011;70: 353–361. doi: 10.1002/ana.22487.

Udeochu, J.C., Shea, J.M., Villeda, S.A. (2016). Microglia communication: Parallels between aging and Alzheimer's disease. Clin Exp Neuroimmunol. 7:114-125. doi: 10.1111/cen3.12307

GRP78 is a new potential therapeutic target for childhood leukemia

Tania Angeles Floriano, Alejandro William Aguirre Guillen, Ricardo Valle Rios

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
Universidad Autónoma Metropolitana, Mexico City, Mexico
tania.angeles@yahoo.com.mx; alejandroaguirre_guillen@hotmail.com; vallerios@gmail.com

Keywords: Leukemia, childhood cancer, GRP78 protein, ISM1, LLA-B

Acute lymphoblastic leukemia (ALL) is the most frequent type of leukemia in pediatrics individuals, being the B-ALL the most common (80–85%) compared with the T-ALL (10–15%). The 5-year survival rate of childhood ALL in developed countries is 90%, while in Mexico it is approximately 67%. The development of treatments against new therapeutic targets is necessary to reduce side effects and improve the quality of life.

GRP78 is a chaperone-type heat shock protein present in the endoplasmic reticulum (ER), which facilitates the folding and assembly of proteins, binding to Ca2 + and regulates stress signaling through the UPR pathway. Its anti-apoptotic role and its role in the survival of tumor cells, tumor progression and angiogenesis, metastasis and resistance to therapy have been described. Interestingly several studies have observed its presence on the surface of cancer cells but not in normal cells opening the question if all kind of cancer express GRP78 and how this may be a checkpoint for cellular homeostasis and importantly if GRP78 might workas a target in cancer. Under this context, it has been reported that ISM1, a 60kDa secretion protein, interacts with GRP78 on the cell surface causing mitochondrial dysfunction that leads to cell death in tumor cells. Currently,our working group have observed that the cell line RS4; 11 of B-ALL, presents relocation of GRP78 in the membrane (0.8%) in comparison with the cell lines Reh (0.18), Jurkat (0.16%), and CCRF (0.16) %). Interestingly, in bone marrow cells from pediatric patients with ALL at diagnosis, the presence of membrane GRP78 is about4–11% compared to a healthy pediatric individual (0.57%), indicating that human leukemia cells relocate GRP78 to the plasma membrane, with bone marrow having a greater frequency. Therefore, our objective is to evaluate the potential role of GRP78 as a therapeutic target in the initiation and development of ALL in pediatric patients, through its interaction with the ISM1 protein.

Levels of proinflammatory cytokines in type 2 diabetes mellitus smokers and non-smokers patients. A preliminary report

Soraya Zavaleta[1], Janeth Oliva Guandorena Gómez[1], Claudia Muñoz Yañez[1], María Aurora Maravilla Dominguez[1], Alondra Martínez Sandoval[1], Amali Muñiz Torres[2], Maria Fernanda Porras[1], Maria De Lourdes Zermeño Gonzalez[3]

[1]Universidad Juárez del Estado de Durango, Durango, Mexico
[2]Hospital General de Gómez Palacio, Durango, Gomez Palacio, Mexico
[3]Unidad Médica para Enfermedades Crónicas, Lerdo, Mexico
zams2109@yahoo.com.mx; janethguangorenagomez@gmail.com; claudiamunoz.yanez@gmail.com; draauroramaravilla@gmail.com; nutricion.alondra@hotmail.com; amalizavaleta@hotmail.com; drasorayazavaleta@gmail.com; maluzer2@gmail.com

Keywords: Insulin Resistance, Smoking, Interleukin 6, proinflammatory cytokines, tumor necrosis factor alpha, interleukin 8, type 2 diabetes mellitus

Introduction: Smoking causes insulin resistance, is associated with type 2 diabetes mellitus (T2DM), and increase in serum levels of interleukin 8 (IL-8) and a persistent inflammatory state (1–3). Tumor necrosis factor Alpha (TNF-α), interleukin 6 (IL-6) and IL-8 are involved in the pathogenesis of T2DM, and play a significant role in inflammatory state, macrophages infiltration and activation in adipose tissue promoting insulin resistance (4, 5).

Objective: to evaluate TN-Fα, IL-6 and IL-8 circulating levels in adult patients with T2DM smokers and non-smokers subjects, and to describe clinical, hematologic and biochemical correlates.

Material and Methods: for this cross-sectional study we included 46 diabetic smokers and non-smokers who attended the Hospital General de Gómez Palacio and the Unidad Médica para Enfermedades Crónicas, Lerdo, Durango, México. The hematological, biochemical and clinical history of the patients were recorded. A blood sample was obtained for the quantification of serum levels of IL-6, IL-8 and TNF-α by the ELISA method.

Results: the average age of the patients was 54.6 years. Others characteristics are shown in the table 1.

The serum levels of IL-8 were 60.6 vs 57.6 pg/mL (p= 0.334); IL-6 levels were 13.9 vs 13.7 pg/mL (p= 0.697); and the levels of TNF-α were 698.3 vs 562.4 pg/mL (p= 0.798), in the group of smokers and non-smokers, respectively (table 2). The total number of platelets and the High Density Lipoprotein cholesterol (HDL) were higher in the non-smoker group than in smokers (data show in table 3).

In the correlation analysis IL-8 was positively associated with HDL-cholesterol (rho = 0.410, p = 0.009) and with TNF-α levels (rho = 0.371, p = 0.014); TNF-α correlates positively with IL-6 levels (rho = 0.326, p = 0.033).

In the non-smokers group the IL-6 correlates positively with TNF-α (rho= 0.494, p= 0.017); TNF-α with serum levels of glucose (rho= 0.447, p=0.037) and the IL-8 with HDL- cholesterol levels (rho=0.515, p= 0.014). In the group of smokers, IL-6 is positively associated with weight (rho = 0.468, p = 0.037); IL-6 and TNF-α with the total number of erythrocytes, hemoglobin and hematocrit and finally the TNF-α with glucose levels (rho = 0.574, p = 0.040). Tables 4 y 5.

Conclusions: Patients with T2DM have high serum levels of IL-6, IL-8 and TNF-α regardless of the smoking habit. The TNF-α/glucose correlation confirms the increase of the insulin resistance. The association of IL-6 and TNF-α with the hematological parameters is probably due to the characteristic poliglobulia (x103/ mm3) of the smoker patients. Further studies are warranted for evaluating the IL-8/HDL positive correlation because other studies report a negative correlation between these parameters.

REFERENCES

1. Bergman BC., et al. (2012), Novel and reversible mechanisms of smoking-induced insulin resistance in humans. Diabetes; 61(12):3156-66. doi: 10.2337/db12-0418.
2. Li Y., et al. (2018)., Impact of Cigarette Smoking on the Relationship between BMI and Insulin: Longitudinal Observation from the Bogalusa Heart Study. Diabetes Obes Metab.. doi: 10.1111/dom.13259.
3. Xu H., et al. (2018). In type 2 diabetes induced by cigarette smoking, activation of p38 MAPK is involved in pancreatic β-cell apoptosis. Environ Sci Pollut Res Int. . doi: 10.1007/s11356-018-1337-3. [Epub ahead of print]
4. Cimini FA., et al. (2017). Circulating IL-8 levels are increased in patients with type 2 diabetes and associated with worse inflammatory and cardiometabolic profile. Acta Diabetol. 54(10):961-967. doi: 10.1007/s00592-017-1039-1.
5. Rehman K., et al. (2017), Role of Interleukin-6 in Development of Insulin Resistance and Type 2 Diabetes Mellitus. Crit Rev Eukaryot Gene Expr. 27(3):229-236. doi: 10.1615/CritRevEukaryotGeneExpr.2017019712.

Activation of CD4 and CD8 TCR-Vβ2+ cells in kawasaki disease shock syndrome

Giselle Quezada Ortega[1], Marco Antonio Yamazaki Nakashimada[2], Marycarmne Godínez-Victoria[1], Mónica Adriana Montoya Guzmán[1], Maria Elisa Drago-Serrano[3], Rafael Campos Rodríguez[1]

[1]Escuela Superior de Medicina (IPN), Mexico City, Mexico
[2]Instituto Nacional de Pediatria, Mexico City, Mexico
[3]Universidad Autónoma Metropolitana, Mexico City, Mexico
qogiselle@outlook.com; yzki71@yahoo.com.mx; maric_27@yahoo.com; monguz22@hotmail.com; dragome@yahoo.com; citlicampos@gmail.com

Keywords: Pediatrics, Shock, Superantigens, kawasaki disease, T cells subpopulations

Background: Kawasaki disease (KD) is a syndrome of unknown etiology manifested by multisystem vasculitis involving medium and small-caliber arteries. Although it is a rare and rare condition, it is the most common cause of acquired heart disease in the pediatric population. Clinically KD encompasses a wide spectrum of manifestations including 3 clinical forms: complete (CKD), incomplete (IKD) and atypical (AKD). Last form comprises the Kawasaki disease shock syndrome (KDSS), with a high frequency of gastrointestinal manifestations and clinical characteristics that are very similar to those found in the toxic shock syndrome caused by a superantigen (SAg). Accordingly the hypothesis of this study was proposed as: the etiology of KDSS is associated with the activation of TCRVβ2 and TCRVβ8 lymphocytes by the presence of SAg's from bacteria that colonize the gastrointestinal tract

Objective: To determine the association between the clinical forms of complete, incomplete and atypical KD and the activation of the TCRVβ2 and Vβ8 lymphocyte

Material and Methods: This was an observational, longitudinal, analytical and prospective clinical study performed in fourty pediatric patients, both sexes with acute KD, admitted in the National Institute of Pediatrics between August 2016 and August 2017. Percentage (%) of CD4 and CD8 T cells expressing TCRVβ2 as well the activated lymphocytes CD25+ were determined in blood samples by flow cytometry. The results were stratified into complete, incomplete and atypical KD.

Results. In comparison to complete KD, KDSS patients have a marked lymphopenia at the expense of CD4+ T cells. Activated T helper and cytotoxic lymphocytes (CD25 +) did not show significant differences but the % of CD4 + TCR VB2 CD25 + T cells was significantly higher. The patients with the IKD form presented a great variability in the % of the lymphoid populations studied without presenting significant differences in regard to the complete and atypical forms

Conclusion: The results of this study did not allow us to accept or reject the hypothesis of this study due to the fact that the number of patients with KDSS was very small. However, these preliminary data suggest that there is an activation of Vβ2 TCR cells that may be activated by a SAg.

Perspective: This study could contribute to complement and strengthen the clinical diagnosis of KD with immunological tests by assessing TCRVβ2 and TCRVβ8 T lymphocyte subpopulations and the presence of SAg's of intestinal bacteria.

Rheumatoid factor combined with anti-modified citrullinated vimentin is associated to risk cardiovascular in rheumatoid arthritis

Iris Paola Guzmán-Guzmán[1], Oscar Zaragoza-García[1], José Eduardo Navarro-Zarza[2], Ileana Torres-Alarcón[1], Mónica Ramiréz[3], Isela Parra-Rojas[1]

[1]Autonomous University of Guerrero, Chilpancingo, Mexico
[2]General Hospital from Chilpancingo, Chilpancingo, Mexico
[3]Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico City, Mexico
pao_nkiller@yahoo.com.mx; zaragoza789@hotmail.com; eduardo@navarrozarza.mx; monse_a12@hotmail.com; ruanomoni@ciencias.unam.mx; iprojas@yahoo.com

Keywords: Rheumatoid Factor, Rheumatoid arthritis, anti-MCV, anti-CCP, Cardiovascular risk

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease, related with excess overall mortality due to increased cardiovascular disease, and characterized by the presence of various autoantibodies in serum. The clinical usefulness of the rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPAs) has been acknowledged due to their acceptable sensitivities and specificities, and prognostic values. Recently, the anti-mutated citrullinated vimentin (anti-MCV) has been associated with risk cardiovascular (RCV) markers (high-sensitivity C reactive protein, IL-6 levels, homeostasis model assessment for insulin resistance (HOMA-IR) index and carotid intima-media thickness). Our objective was evaluate the combined class of autoantibodies ((none, one or two present (Anti-CCP, anti-MCV and RF)) with RCV markers, in Mexican patients with rheumatoid arthritis. The positivity to RF, Anti-MCV and anti-CCP were determinate in 195 patients with RA. Serum levels of high sensitivity C-reactive protein (hsCRP) and atherogenic indices; Castelli, Kannel, TGs/HDL-c were consider to define CVR in the patients. In this study 74.9% patients was triple-positive, whereas 7.7% were Anti-CCP+/Anti-MCV+/RF negative; 6.2% Anti-CCP negative/Anti-MCV+ /RF+; and 4.1% Anti-CCP negative/Anti-MCV negative/RF+. Patients with double-autoantibody-positive (Anti-MCV and FR) showed RCV increased according to Kannel index (>3%) (OR=4.0, IC95% 0.62–27.2, p=0.08), Castelli (>4.5% in male, >5% in female) (OR=2.6, IC95% 0.4–20.5, p=0.24), and TG/HDL index (>3%) (OR=2.6, IC95% 0.4–20.5, p=0.24), compared with triple-positive patients and double-autoantibody-negative ACPAs. The positivity to RF combined with Anti-MCV is associated with RCV increased in rheumatoid arthritis patients.

Patients adults with common variable Immunodeficiency and their association with Freiburg classification and clinical manifestations

Diego Antonio Mendoza Revilla, Patricia M. O'Farrill Romanillos, Nora Hilda Segura Méndez

Centro Medico Nacional Siglo XXI, Mexico City, Mexico
da.mr@hotmail.com; patyfritzenwalden@hotmail.com; norasegura@yahoo.com

Keywords: Common Variable Immunodeficiency, phenotypes, Adults patients, Classification Freiburg, Criteries diagnostics

Introduction: The Common Variable Immunodeficiency (CVID) comprises the Immunodeficiency Primary symptomatic most common in the world, it is a heterogeneous disease of hypogammaglobulinemia with distinct phenotypes clinics whose predispose to recurrent infections, a higher incidence of autoimmunity, and malignancy. The memory B cells (memBcs) are key players in humoral defense and their numbers are commonly reduced in these patients.

Objective: Correlate the clinic of adult patients with CVID according to the classification by phenotypes.

Material and Methods: We studied 18 patients from a cohort, with a diagnosis of CVID, of the Immunodeficiency Clinic of the Specialty Hospital of National Medical Center Siglo XXI. Flow cytometry was performed with determination of lymphocyte subpopulations with absolute and relative numbers, including: CD19, CD27 + IgM-IgD-, CD21 low. Patients were grouped according to the classification of Freiburg into 3 types: Ia, Ib, II, according to their values of LB CD27 + IgM-IgD-, and LB CD21 low.

Results: 7 male and 11 female, current average age of 42 years. 8 patients with autoimmune diseases, all hematologic, the most common Immune Thrombocytopenic Purpura. 7 with IDCV-associated enteropathy. 1 with Chronic Myeloid Leukemia and 3 with predominance of infectious processes.

According to the classification of Freiburg, 4 patients with group Ia, 4 Ib and 10 with group II.

75% of patients with Ia and Ib groups had hematological autoimmune diseases compared to 20% of those in group II.

40% of patients in group II had predominance of infections and 30% of IDCV-associated enteropathy.

Also, 4 patients died, 2 due to massive lower gastrointestinal bleeding, 1 due to septic shock and one due to suicide.

Conclusion: In this study, patients with less than 0.4% of isotype-memory LB were associated with hematological autoimmunity, and those with more than 0.4% of CD27 + IgM-IgD- showed greater frequency of enteropathy and predominance of infectious processes. According to the B cell subtypes and their classification, it is possible to find a marker that identifies the genotype of the disease and its related clinical presentation.

Changes at the pulmonary level in patient with variable common immunodeficiency

Irving Jesus Vivas Rosales, Mariana Hernandez Ojeda, Patricia Maria O`Farrill Romanillos, Nora Hilda Segura Mendez

Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
irvingvivas@gmail.com; mhdezo@hotmail.com; patyfritzenwalden@hotmail.com; norasegura@yahoo.com

Keywords: Adult, Bronchiectasis, Common Variable Immunodeficiency, Tomography, X-Ray Computed, immunodeficiency

Background: Pulmonary parenchyma can be affected in patients with variable common immunodeficiency. This immunodeficiency is the most common primary symptomatic humoral immunodeficiency in adults. Pulmonary complications occur in 27–34%, may be infectious or non-infectious. Of the former, bronchiectasis are permanent dilatations of the bronchi, its prevalence is up to 70%, however there is little information regarding the type and location of the same. The most common noninfectious cause is pulmonary interstitial disease (PID) in 15–20%, suggesting its presence in voided glass, bilateral septal thickening, parahilar and mediastinal lymphadenopathy.

Methodology: It has been made a transversal, observational and descriptive study that included 32 adult patients with diagnosis of CVID according to the criteria of the European Society of Immunodeficiencies (ESID). The population included both genders, older than 18 years who had high resolution chest X-ray (CCT), interpreted by the same expert radiologist. Patients with infectious process were excluded.

Results: We included 32 patients, 10 (31.2%) men and 22 (68.7%) women, mean age 42 ± 17 years. 40.6% had bronchiectasis, 23% had a lobe involvement, 15.3% two lobes, 46.1% 3 lobes and 15.3% complete involvement of the parenchyma. The types of bronchiectasis were distributed as follows: tubular 38.4%, varicose 23% and cystic and tubular combinations 15.3%, cystic and varicose 15.3% and cystic, tubular and varicose 7.6%; other pulmonary findings were atelectasis, fibrosis, subsolid nodules, calcifying granulomas, and lymphadenopathy. In those without bronchiectasis (59.4%), other manifestations were linear pathways of fibrosis, calcified granulomas, interstitial thickening, subsolid nodules, bulls and diaphragmatic elevation.

Conclusion: Changes that occur in patients with CVID may be variable and include bronchiectasis and alterations in both the parenchyma and the interstitium. Timely detection is important to improve the prognosis in this group. It has been reported that patients with CVID and PID have an average survival of 13.7 years vs. 28.8 years of those without PID. The tomographic findings suggesting PID suggest the need for pulmonary extension studies. Timely diagnosis is imperative, as these findings may be part of lymphocytic granulomatous lung disease, this findings require effective treatment, since they worsen the prognosis and quality of life of patients.

Characteristic responses of CD38+ central memory CD4+ T cells to TCR engagement suggest a decreased survival capacity

Jessica Romero Rodríguez[1], Gustavo Olvera-García[1], Fernanda Cervantes Mejía[1], Damaris. P. Romero-Rodríguez[2], Eduardo Montes[3], Joel Vázquez-Pérez[4], Enrique Espinosa[1]

[1]Integrative Immunology Laboratory, National Institute of Respiratory Diseases (INER), Mexico
[2]Cytometry Chore Facility, INER, Mexico
[3]Molecular Biology Laboratory, INER, Mexico
[4]Virology Department, INER, Mexico
joy_tavo288@hotmail.es; hector.enrique.espinosa@gmail.com

Keywords: Cell Death, HIV Infections, CD38, Ectoenzyme, Differential expression of genes, Centrall memory T CD4+ cells

Introduction: Evidence from animal models and humans suggests that central memory CD4+ T (TCM) cells sustain the chronic phase of HIV infection by maintaining a residual effector memory (TEM) CD4+ T cell population. TCM cells gradually lose their homeostatic capacity. The cause of this loss is currently unknown.

A great percentage of TCM cells from HIV+ patients express the ectoenzyme CD38, and increased CD38 expression on T cells is strongly and independently associated with CD4 T cell loss. It is currently unknown if CD38 reflects a pathogenic mechanism HIV or if it participates directly in it.

Methods: To determine if the gene expression signature of TCM cell in HIV infection (Olvera-García 2016) is associated with CD38 expression, we compared the expression of 83 genes from this signature between CD38+and CD38-TCM cells from 6 healthy donors, under unstimulated and TCR-stimulated conditions, using multiplex PCR. Predictions of protein-protein interactions were performed using String.

Results: We found 20 genes with significantly higher expression in CD38- cells compared with CD38+ after TCR engagement. Possible interactions between 10 of these genes (IL-2,CD40L, DNAJA3, GADD45B, SOD2, HSPB1,MAP3K8, IFN-g, PMAIP1 and MYC) yielded a predicted regulation of apoptotic processes (FDR= 0.000295), as

well as programmed cell death (excluding DNAJA3, FDR=0.00105). Positive regulation of cytokine expression, and stress responses (FDR=0.00105) were also predicted. Most genes supporting these predictions were overlapping.

Conclusion: The TCM cell gene expression signature in HIV is partly related to CD38 expression. The differential expression of genes between CD38+ and CD38- TCM cells suggests that the latter might be less likely to undergo cell death after TCR engagement. A possible role of the ectoenzyme CD38 should be investigated.

Bronchial asthma as risk factor of dengue severe disease

Ana B. Perez, Beatriz Sierra, Gissel Garcia, Eglis Aguirre, Mayling Alvarez, Maria G. Guzman

Instituto de Medicina Tropical "Pedro Kouri", Havana, Cuba
anab@ipk.sld.cu; siebet@ipk.sld.cu; gmd@ipk.sld.cu; eglis@ipk.sld.cu; mayling@ipk.sld.cu; lupe@ipk.sld.cu

Keywords: Cytokines, Dengue, immune response, Risk factors, bronchial asthma

Introduction: Bronchial asthma has been recognized as a risk factor for the severest form of dengue virus infection: Dengue Hemorrhagic Fever / Dengue Shock Syndrome (DHF/DSS). Dengue is a mosquito-borne tropical disease caused by a flavivirus that is subdivided into four major serotypes (dengue 1, dengue 2, dengue 3 and dengue 4). Immunity to a given dengue virus serotype provides protection against reinfection with that same serotype (homotypic infection). However, subsequent infection with other serotype (heterotypic infection) markedly increases the risk for DHF. In Cuban outbreaks of dengue where sere isolated different viral serotypes (dengue 2, dengue 3 and dengue 4, asthma has been significantly associated with severity and fatal cases.

Objective of the Study: The objective of the present study was to clarify the possible role of cellular immune response, and more specifically of cytokines and cytotoxic mediators, in the association between asthma and dengue. According to this, the expression of some cellular immune response markers induced by dengue virus in PMBC collected from dengue immune asthmatic and healthy control individuals was determined.

Materials and Methods: The presence of serum anti-dengue antibodies was determined by ELISA in 282 individuals. To those individuals who had dengue-specific antibodies, we determined the presence of serum neutralizing antibodies against each viral serotype via neutralization assay. 26 dengue-immune individuals diagnosed as asthmatic patients and 18 healthy controls, all with anti-dengue neutralizing antibodies. PBMC were challenged with dengue 1, 2 or 3 viruses and was quantified the gene expression of Tumor necrosis factor alpha (TNFα), Interferon gamma (IFNγ), Interleukin (IL) 10, IL-4, Transforming growth factor beta 1 (TGFβ1), granzyme B, perforin genes and the housekeeping gene hypoxanthine phosphoribosyltransferase 1 (HPRT-1) and compared between asthmatic and controls.

Results: Statistically significant differences (Mann Whitney test, $p < 0.05$) were observed in the IL-10, TGFb, granzyme B and perforin gene expression between both groups when cells were incubated with different viral serotypes to which the individuals were immune (heterotypic challenge). The lower expression of these four genes was observed in PBMC from asthmatic individuals. Not significant differences in genes expression between PBMC from asthmatic and control individuals were found in homotypic viral challenge, excepting IL-10 gene expression, which showed an increased level in PBMC from asthmatic group ($p = 0{,}004$). Undetectable IL-4 gene expression was observed in all the cases.

Conclusions: The present study, in concordance with previous findings, suggest the importance of anti-inflammatory and cytotoxic response in the control of dengue infection, and provides an initial framework for defining the role of bronchial asthma as risk factor for the severest clinical form of dengue.

Role of NF-kB and lipids metabolism in the African-ancestry protection against Dengue Haemorrhagic Fever

Beatriz Sierra, Gissel Garcia, Ana B. Perez, Eglis Aguirre, Mayling Alvarez, Maria G Guzman

Instituto de Medicina Tropical "Pedro Kouri", Havana, Cuba
siebet@ipk.sld.cu; gisselgarcia@infomed.sld.cu; anab@ipk.sld.cu; eglis@ipk.sld.cu; mayling@ipk.sld.cu; lupe@ipk.sld.cu

Keywords: Dengue, Genomics, NFkB, Lipids metabolism, ancestry informative SNPs

Introduction: Individuals can display differential genetic susceptibility to infectious diseases. The arthropod-born viral dengue disease is one such disease. Global ancestry analysis based on high-throughput genotyping in admixed populations can be used to test this hypothesis, while admixture mapping can map candidate protective genes.

Materials and Methods: A Cuban dengue fever cohort was genotyped using a 2.5million SNP chip. Global ancestry was ascertained through ADMIXTURE and used in a fine-matched corrected association study. A gene set enrichment analysis of candidate genes was performed in dengue transcriptome in whole blood as a surrogate of the LXR/RXR interaction pathway in macrophages.

Results: We demonstrated that OSBPL10, LDLR and MSR1, mediating lipid metabolism, and RXRA, NF-kB, mediating cytokines production, were always upregulated in the convalescents dengue patients or, biologically more meaningful, down regulated in dengue hemorrhagic fever patients, with main genes been RXRA and NF-kB. We also demonstrated that both genes interact in the LXR/RXR activation pathway macrophages; that integrates lipid metabolism and immune functions, being a key player in dengue virus entrance into cells, its replication therein and in cytokine production.

Conclusion: This analysis reinforces the importance in dengue disease of the enlarged pathway for LXR/RXR activation, including the cholesterol/lipids metabolism and the NF-kB control of cytokines.

Role of SOCS3, SHP1-2 and PIAS3 proteins on the activation of STAT3 in macrophages during infection with Taenia crassiceps

Sandy Reyes Martínez, Yadira Ledesma-Soto, Yolanda González Hernández, Cuauhtémoc Del Ángel Sánchez Barrera, Blanca Estela Callejas Piña, Ilse Chávez Soto, Luis Ignacio Terrazas Valdés

Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla de Baz, Mexico
sandy_reyes25@comunidad.unam.mx; lesy_790413@yahoo.com.mx; yolandagonzaleshdz@gmail.com; angel.casb@hotmail.com; aletse_bianca@hotmail.com; ilsebios01@gmail.com; literrazas@campus.iztacala.unam.mx

Keywords: Macrophages, STAT3 Transcription Factor, socs3, Taenia crassiceps, SHP-1 and SHP-2 phosphatases

Helminth parasites are eukaryotic organisms with the ability to regulate and modulate the immune response in their hosts. The strategies involved to assure the establishment and survival of these parasites have been the subject of study in recent decades. However, the mechanisms that helminths use are not yet fully understood. Helminth infections and the host immune responses are products of a prolonged dynamic co-evolution between the host and the parasite, such mechanisms of regulation are thought to establish a balanced immunological state in the host, often termed as a modified T helper type 2 (Th2) response that allows a long reproductive phase of the parasites. This response is a feature of helminth infection and Taenia crassiceps is not the exception. Several evidences have suggested that alternatively activated macrophages (aaM), induced by Th2 type responses, help to create an anti-inflammatory environment favorable for parasite survival. In part, this is possible thanks to the activation and regulation of specific molecules that participate in the signaling of multiple cytokines and growth factors.

T. crassiceps infection and his excreted/secreted products (TcES) can modulate specifically the JAK-STAT pathway. Also, in previous studies by our group it has been observed that macrophages from T. crassiceps-infected mice expressed high levels of SOCS3 and the STAT3 protein showed different phosphorylation responses with the cytokines IL-6 and IL-10.

In this research, we set out to study whether infection with T. crassiceps would induce different activation patterns of the SOCS3, SHP1-2 and PIAS3 regulatory proteins

that modulate STAT3 activation. This study was carried out in a murine model. Six to eight week-old female BALB/c mice were infected with an intraperitoneal (i.p.) injection of 20 small non-budding cysticerci of T. crassiceps. The peritoneal exudate cells (PECs) were obtained from the uninfected mice, as well as from 2 and 8 weeks T. crassiceps-infected mice. After 24 h in culture, non-adherent cells were removed and the remaining adherent cells were stimulated by addition of recombinant murine IL-6 and IL-10 (20 ng/mL) for 20 min and lysed with cold lysis buffer. Phosphatase inhibition by orthovanadate was performed adding 20 μM of sodium orthovanadate 45 minutes or with 10-5M Zoledronic acid (ZA) for 16–18 h previously to cell stimulus. The protein concentration was determined with Bradford assay and stored at -70°C until further use. Western blot analysis was performed to analyze protein levels of SOCS3, phospho-SHP1, phospho-SHP2, PIAS3 and phospho-STAT3.

We found that in response to IL-6 and IL-10 macrophages obtained from acute and chronic T. crassiceps-infected mice expressed high levels of SOCS3, phospho-SHP1, phospho-SHP2 and phospho-STAT3. Nevertheless, the inhibition of phosphatase activity of SHP2 by orthovanadate restored the macrophage response to IL-6 and IL-10, by increasing STAT3 phosphorylation without affecting SOCS3 expression. These data suggest that STAT3 phosphorylation is regulated by phosphatases instead of SOCS3. On the other hand, the macrophages obtained from acute and chronic infections expressed high levels of the protein PIAS3 compared to macrophages from naïve mice. Furthermore, macrophages from T. crassiceps-infected mice exposed to Zoledronic acid (ZA) expressed low levels of SOCS3 as well as phospho-STAT3. The exposure to this drug showed that the effect of the decrease in SOCS3 protein levels was probably in response to the decrease of the STAT3 phosphorylation. Together these data suggest that helminth infection may target intracellular pathways to manipulate macrophage response to cytokines such as IL-6/IL-10.

Immune response against dengue and zika infection

Ana B. Perez, Beatriz Sierra, Eglis Aguirre, Claudia Bracho, Mayling Alvarez, Pedro A. Martinez, Daylin Medero, Naifi Calzada, Maria G. Guzman

Instituto de Medicina Tropical "Pedro Kourí", Havana, Cuba
anab@ipk.sld.cu; siebet@ipk.sld.cu; eglis@ipk.sld.cu; bracho@ipk.sld.cu; mayling@ipk.sld.cu; pamartinez@ipk.sld.cu; daylin@ipk.sld.cu; naifi@ipk.sld.cu; lupe@ipk.sld.cu

Keywords: Dengue, immune response, disease progression, T cells subpopulations, Zika

Introduction: Dengue virus (DENV) and Zika virus (ZIKV) are flavivirus which have been spreading during the last three years in the same American countries. DENV includes four distinct viral serotypes and it is estimated that the annual global incidence is 390 million cases. Most clinical DENV infections result in a self-limiting febrile illness but can result in a severe disease characterized by plasma leakage, which can lead to shock syndrome, with mortality rate of up to 20% of affected individuals. On the other hand, Zika became the first major infectious disease linked to human birth defects to be discovered in more than half a century and created such global alarm that the World Health Organization would declare a Public Health Emergency of International Concern. There is a wide degree of cross-reactivity in the immune response against those flaviviruses because of the homology in the amino acid sequence of their proteins. The role of this fact in cross-protection or diseases pathogenesis is a priority for the design of vaccines against those infections.

Objective of the Study: The characterization and comparation of humoral and cellular immune response during DENV and ZIKV infections in humans is the main aim of this study.

Materials and Methods: PBMC subsets frequencies and their activation were analyzed by flow cytometry and evaluated their correlation with antibodies levels, viral load and clinical outcome in patients with DENV or ZIKV infections during acute phase of the disease.

Results: The frequency of activated T CD8+ cells and memory T cells was significantly higher in sequential than primary DENV infection. Warning signs and complications predominated in DENV patients during secondary infection. The occurrence of warning signs in patients was associated with higher frequency of activated cells. Mucosal bleeding and persistent abdominal pain were associated with increased frequency of specific cell subsets. An increased CD4+ T cells activation was detected in DENV comparing with ZIKV infection. Wide CD8+ T cells activation was shown during ZIKV infection. Specific and cross-reactive antibodies were detected after sixth day after DENV and ZIKV infection. DENV and ZIKV loads and antibodies levels were correlated with frequencies of activated PBMC. Differences were detected between primary and secondary heterotypic flavivirus infections.

Conclusions: The elucidation of the role of the immunological mechanisms in dengue and zika pathogenesis is a priority not only for the illness treatment, but also for the evaluation of vaccine candidates against those flavivirus. Our findings suggest that activation of some specific T cells subsets may be involved in the recovering but also in the development of complications like bleeding or plasma leakage during dengue disease, contributing with the better understanding of disease pathogenesis. These results aid to clarify the role of cellular immune response in the control of DENV and ZIKV infections. Higher pro-inflammatory response in DEN disease and higher cytotoxic response in ZIK disease could explain the clinical course of these infections in humans.

Bovine tuberculosis biomarkers identification using microarray technologies

Genesis Adrian Mayorquin[1], Jonathan Aguilar[2], Saret Figueroa[2], Javier Jovany Sanchez[2], Hilda Linda Perez[3], Gloria Guillermina Guerrero Manriquez[2]

[1]Unidad Profesional Interdisciplinaria de Ingeniería Zacatecas (IPN), Zacatecas, Mexico
[2]Autonomous University of Zacatecas, Zacatecas, Mexico
[3]Departamento de Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
genesis.ipn@gmail.com; jony.sd@hotmail.com; sareth310594@gmail.com; jovanykill@hotmail.com; hlperez@hotmail.com; gloguerrero9@gmail.com

Keywords: M. bovis, Biomarcadores, Tuberculosis bovina, expresion genica, Microarreglos, vias de señalizacion

Bovine tuberculosis (BTb) is a cronic disease of cattles, caused by Mycobacterium bovis, a gram-positive bacteria, member of the Mycobacterim complex, and the Mycobacteriaceae family (Grange JM., 2001; Cassidy., 2006). Bovine tuberculosis of zoonotic importance constitute a serious animal heatlh problem (Thoen et al., 2006; Waters et al., 2011; WHO., 20012). It estimated that more than 50 millions of cattles are infected with M. bovis, causing economical losses in agriculture and livestock (Garnier et al., 2003; Hewinson et al., 2006). M. bovis is transmitted mostly by aerosol (80–90%) but it can transmitted by oral route (¯meat or unpausterized milk) (Abalos and Retamal., 2004; Biet et al., 2005).). The risk of transmitting bTB to humans in Mexico might be explained by the high prevalence of the disease in cattles (16%), the manipulation of infected animals (a common practice in Mexico) and the consumption of the unpasteurized milk. Accordingly to a recent report from the Binational Organism Mexico-EUA (SAGARPA., 2012), 82% of National territory is maintained a prevalence of 0.5% of bTB except in the zones of dairy livestock; while there are other regions where the prevalence is unknown or even higher, (Millian et al., 2000; Zendejas., 2007). Tuberculosis in humans is caused mostly by M. tuberculosis, but also can be the effect of other mycobacterias like M. bovis. It is more frequent to find M. bovis in humans than M. tuberculosis in livestock like cattles (Phillips et al., 2002). Humans or bovines are able to induce strong immune responses, characterized by IFN-γ production o TH1, after M. tuberculosis ó M. bovis infection and develop active disease (Ritacco et al., 1991; Flynn and Ernst., 2000; Waters y cols., 2011). Indeed, it is accepted that cellular immune response play a key role against pathogenic mycobacteria, characterized by interferon gamma production (IFN-γ) derived from the CD4+ T cells (Flynn and Chan., 2001), which are important for either to contain the tubercle bacilli as well as to keep

the activated state of macrophages (Tascon et al., 1998). Nowdays, it has been shown that in the last stages of the infection, CD8+T cells are also playing a key role (Liebanna et al., 1999; Cooper et al., 2009) either in the human or bovine tuberculosis caused by M. tuberculosis or M. bovis. Moreover, the induction of cellular immune responses type Th1/TH17 it seems that are important for the protection against tuberculosis (Bhuju et al., 2012). A better understanding of these protective antimuycobacterial cellular immune responses are required in order to develop more effective vaccines and diagnostic tests (Koul et al., 2004). Indeed, there is very few reports about to find immunological correlates of protection which would improve vaccine development (Hewinson et al., 2003; Buddle et al., 2005. Thus, the application, of the microarray tecnologies is a tool very usable to identify biomarkers that correlate the progression of the pathology and define the different stage of the infection which could have impact on the rational design of novel diagnostic approaches (Mortazari et al., 2008; Berry et al., 2010; Aranday-Cortes et al., 2012¸ Bhuju et al., 2012; Malone & Oliver., 2011). The quantitative tools that measure changes in the expression of genes have advanced enormously due to the necessity to find methods more effective, sensible, faster and that be possible to determine a more integrated response of the interaction host-pathogen (Livak & Schmingen., 2001; Malone & Oliver, 2011). Two recent studies demonstrated the potential of the microarray technology as a molecular diagnostic method but also it allows to identify biomarkers with therapeutic potential in human TB (Berry et al., 2010) or bovine Tuberculosis (Bhuju et al., 2012; Golby et al.., 2014; Pirson et al., 2015; Conlan et al., 2015). Bhuju et al. (2012), by making a transcriptome analysis in vaccinated cattle, demonstrated that there is a dominant role of IL-22 for protection against TB. In a different experimental settings, Aranday-Cortes et al., 2012, using a mouse model of M. bovis infection, tried to apply the microarray technologies in combination with the murine M. bovis infections experiments to select the most strongly up-regulated genes expressed from the whole transcriptome of lung and spleen cells to predict biomarkers of disease in M. bovis infected cattle. In the present work, it is reported the genic expression profiles from the RNA extracted from total blood of M. bovis infected cattles and compared with negative control animals from the state of Zacatecas and from the nearby regions. Normalization and filtering analysis revealed that there is a pool of genes that are up-regulated mostly involved in binding to different components like to ions, to cofactors, to Calmodulin, and Tubulin. Others genes participate on the activity of ATP. While another set of genes that are down regulated are related to receptor activities such as Manose, and Prostaglandina E receptor; genes that participate as mediators of the vesicular transport, of the lipid phosphatase, and/or Calmodulin 3 activity. All the data suggest that through the application of the microarray technologies is possible to identify biomarkers that could define stages of the infection disease in cattles; with the aim to develop an alternative diagnostic method with more sensibility to discard between negative and positive false.

REFERENCES

Grange JM. 2001. Tuberculosis (Edinb) 81: 71-77

Cassidy JP. 2006. Vet Microbiol 112: 151-161

Garnier T, Eiglmeier K, Camus JC, Medina N, Mansoor H y cols. 2003. Proc. Natl. Acad. Sci USA 100: 7877-7882

Hewinson RG, Vordemeier HM, Smith NH, Gordon SV. 2006. Vet Microbiol. 112: 127-139

Waters WR, Palmer MV, Thacker TC, Davis WC, Sreevatsan S et aly. 2011.. Clinical Developmental Immunology

World Organization for Animal Health. 2012.- Página web: www.oie.int/wahid-prod/ public.php?page=disease_status_detail&disease_id=32

Thoen Ch., LoBue P.A. & de Kantor I.N. 2006. Vet. Microbiol., 112 (2-4), 339-345

Milian Suazo F, Sánchez LM, Toledo P, Ramírez C, Santillán M.A. (2000).- Rev. Latinoam. Microbiol., 42: 13-19

Abalos P, P Retamal. 2004. Rev. Sci. Tech. Off. Int Epiz 23: 583-594

Biet F, Boschiroli ML, Thorel MF, LA Guilloteau. 2005. Vet. Res. 36: 411-436

Zendejas Martínez H, Milián Suazo F, Cuador Gil Q, Cruz Bello G, Anaya Escalera A.M, Huitron Marquez G, García Casanova L. 2007. Vet. Ital., 43 (3), 629-634

Phillips CJ, Foster CR, Morris PA, R Teverson. 2002.. Anim. Health. Res. Rev. 9: 3-13

Ritacco V, Lopez B, De Kantor IN, Barrera I, Errico F, y col. 1991.. Res Vet Sci 50: 365-367

Flynn JL, JD Ernst. 2000. Curr. Op. Immunol. 12: 432-436

Liébana E, Girvin MR, Welsh M., Neill DS, MJ Pollock. 1999. Infec. and Imm. 67: 1034-1044

Flynn JL , J Chan. 2001.. Annu. Rev. Immunol. 19: 93-120

Tascon RE, S, Stavropoulos, Lukacs KV, MJ Colston. 1998. Infect. Immun. 66: 830-834

Cooper AM. 2009. Annu Rev. Immunol. 27: 393-422

Koul A, Herget T, Klebl B, Ulrich A. 2004. Nat. Rev. Microbiol. 2: 189-202

Hewinson RG, Vordemeier HM, BM Buddle. 2003. Tuberculosis (Edinb) 83: 119-130

Buddle BM, Wedlock DN, Denis M, Skinner MA. 2005. Vet Immunopathol 108: 45-51

Villarreal-Ramos B, Berg S, Chamberlain L, McShane H, Hewinson RG, Clifford D, Vordermeier M. 2014. . Vaccine. 32: 5645-9

Bhuju S, Aranday-Cortes E, Villareal-Ramos B, Xing Z, Singh M, HM Vordemeier. 2012. PLOS One. 8: e1003077

Mortazavi A, Williams BA, McCue K, Schaffer L, Wold B. 2008.. Nat. Methods 5: 621-628

Aranday-Cortes E, Hogarty PJ, Kaveh DA, Whelan AO, Villareal-Ramos B, y col. 2012. PLoS One 7: e30620

Berry PRM, Graham MCh, McNab WF, Xu Z, Bloch AAS, Oni T, Wilkinson AK, Banchereau R, Skinner J, Wilkinson JR, Quinn CH, Blankenship D, Dhawan R, Cush J, Mejias A, Ramilo Ó, Kon MO, Pascual V, Banchereau J, Chaussabel D, and A Ó'Garra. 2010. Letter to Nature. 466: 973-979

Malone JH, Oliver B. 2011. BMC Biol 9: 34

The role of Syk and STAT3 in the regulation of Interleukin 10 expression in human macrophages infected with Mycobacterium bovis

Edgardo Madrid-Paulino, Tomás Villaseñor, Rafael Maldonado-Bravo, Leonor Pérez-Martínez, Gustavo Pedraza-Alva

Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
edgardo_madrid460@hotmail.com; tomas@ibt.unam.mx; rafamb@ibt.unam.mx; leonor@ibt.unam.mx; gustavo@ibt.unam.mx

Keywords: IL-10, PKC, stat3, Syk, M. bovis

Mycobacterium tuberculosis is the cause of human tuberculosis. In 2015 10.4 million cases of tuberculosis were recorded and 784 000 persons died for this disease. Mtb increased propagation among individual with acquired immunodeficiency syndrome and the appearance of drug resistant strains represent a world-wide serious health problem (WHO 2016). In most cases, the immune response against Mtb is sufficient to avoid developing active disease; however, complete destruction of the pathogen is frequently not achieved. Macrophages contribute to the elimination of bacilli via numerous mechanisms, including the successful acidification and maturation of phagosomes (Murray and Wynn 2011). However, Mtb ensures its survival within host macrophages by arresting the maturation pathway that leads to phagosome lysosome fusion (Crevel, Ottenhoff, and Meer 2002). Although it is well known that INFγ produced by T lymphocytes plays a key role against Mtb infection, Mtb promotes the expression of the cytokines IL-10 and TGFβ that antagonize INFγ production and functions, thus altering the normal immune response allowing successful infection (Rosenzweig and Holland, 2005). The signal transduction pathways activated by M. tuberculosis leading to IL-10 and TGFβ expression in macrophages, are poorly understood. Transcriptomic analysis of organs from M. tuberculosis infected animals showed that the activation of the Signal Transducers and Activators of Transcription (STAT) factor is regulated in response to M. tuberculosis infection (Koo, Subbian, and Kaplan 2012; Subbian et al. 2013). The STAT family of transcription factors consists of 7 members (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6) whose activity is regulated by phosphorylation. In the canonical pathway, phosphorylation of STAT is carried out by a tyrosine kinase known as Janus Kinase (JAK), which is activated by different cytokine receptors (Hovarth et al. 2000; Shuai et al. 2003). The STAT proteins after being phosphorylated in the cytoplasm, form dimers that translocate to the nucleus. However, the transcriptional activity of STAT1 and STAT3 is increased when these proteins are within the nucleus and phosphorylated at serine residues by Mitogen-Activated Protein

(MAP) kinases, such as Extracellular Signal-Regulated Kinase (ERK) and p38 (Chung et al. 1997; Decker and Kovarik 2000). Given that i) it has been reported that STAT3 can be phosphorylated and activated by the Spleen Tyrosine Kinase (SYK) (Uckun et al. 2010); ii) that Syk activation is regulated during M. tuberculosis(Marakalala, Graham, and Brown 2010) iii)STAT3 regulates IL-10 expression in B-cells (Moore et al. 2001) and iv) STAT3 phosphorylation increase by stimulating THP-1 macrophages with protein extracts of Mycobacterium bovis (Martinez-Neri et al. 2015) we speculated that mycobacterium activates an alternative signaling transduction pathway involving Syk and STAT3 leading to IL-10 expression.

Here we report that M. bovis BCG-induced IL-10 expression in THP-1 macrophages, involves STAT3 activation and a feedback loop between the protein kinase C (PKC) and Syk.

REFERENCES

Crevel, R., H M O T., and Van Der Meer J. 2002. "Innate Immunity to Mycobacterium Tuberculosis." 15(2): 294–309.

Decker, T., and Kovarik, P. 2000. Serine phosphorylation of STATs. Oncogene 19, 2628–2637.

Horvath, C.M. 2000. STAT proteins and transcriptional responses to extracellular signals. Trends Biochem. Sci. 25, 496–502.

Koo, M., Subbian S., and Kaplan G. 2012. "Strain Specific Transcriptional Response in Mycobacterium Tuberculosis Infected Macrophages." Cell Communication and Signaling 10(1): 2. http://biosignaling.biomedcentral.com/articles/10.1186/1478-811X-10-2.

Marakalala,M., Graham, L., and Brown G. 2010. "The Role of Syk / CARD9-Coupled C-Type Lectin Receptors in Immunity to Mycobacterium Tuberculosis Infections."

Martínez-Neri, P. a., López-Rincón, G., Mancilla-Jiménez, R., del Toro Arreola, S., Muñoz-Valle, J.F., Fafutis-Morris, M., Bueno-Topete, M.R., Estrada-Chávez, C., and Pereira-Suárez, A.L. (2015). Prolactin modulates cytokine production induced by culture filtrate proteins of M. bovis through different signaling mechanisms in THP1 cells. Cytokine 71, 38–44.

Moore, K.W., Malefyt, R.D.W., Robert, L., and Garra, A.O. (2001). I Nterleukin -10 and the I Nterleukin -10. Mol. Cell. Biol. 1, 683–765.

Murray, P.J., and Wynn, T. a (2011). Protective and pathogenic functions of macrophage subsets. Nat. Rev. Immunol. 11, 723–737. Rosenzweig, S.D., and Holland, S.M. (2005). Defects in the interferon-gamma and interleukin-12 pathways. Immunol. Rev. 203, 38–47.

Shuai, K., and Liu, B. (2003a). Regulation of JAK-STAT signalling in the immune system. Nat. Rev. Immunol. 3, 900–911.

Subbian, S., Nirmalya B., Tsenova L., O'Brien P., Khetani V., Kushner N., Peixoto B., Soteropoulos P., . Bader J., Karakousis P., Fallows D, and Kaplan G. 2013. "Early Innate Immunity Determines Outcome of Mycobacterium Tuberculosis Pulmonary Infection in Rabbits." Cell Communication and Signaling 11(1): 1. Cell Communication and Signaling.

IL-23, IL-23Rs, IL-17A and IL-17RAs levels in plasma from patients with Rheumatoid arthritis and Chronic periodontitis

Ruth Rodriguez -Montaño[1], Ana Guilaisne Bernard-Medina[2], Alondra Carmen Ruiz-Gutierrez[1], Edith Oregon-Romero[1], Ramon Franco-Topete[1], Celia Guerrero-Velazquez[1]

[1]University of Guadalajara, Guadalajara, Mexico
[2]Hospital Civil de Guadalajara, Guadalajara, Mexico
ruth_rodriguez21@outlook.com; guilaisne@gmail.com; alondra_crg@hotmail.com; oregon_edith@hotmail.com; francotra@yahoo.com; celiagv2001@yahoo.com.mx

Keywords: Rheumatoid arthritis, IL-23/IL-17, Chroni periodontitis, IL-23R soluble, IL-17RA soluble

Introduction: Rheumatoid Arthritis (RA) is a chronic, systemic and autoimmune inflammatory disease, characterized by inflammation of the synovial membrane, progressive destruction of articular cartilage and bone (1). On the other hand, periodontal diseases (PD) are a group of disorders with different etiologies and clinical manifestations. They include periodontitis, wich is distinguished by a strong inflammatory response. Two main forms of periodontitis are identified; chronic periodontitis (CP) and aggressive (AP), and they are characterized by gingival inflammation, formation of periodontal pockets, attachment loss and alveolar bone destruction (2).

On the other hand, RA appears to be associated with PD due to Porphyromonas gingivalis, and expressing PADI4, which is capable of promoting citrullination of proteins in mammalian. This phenomenon can occur in the joints as in the periodontium (3). Furthermore, lipopolysaccharide (LPS) and other components of the periodontal pathogenic bacteria are recognized by dendritic cells secrete cytokines like IL-1, TGF-β, IL-6 and IL-23. IL-23 binds to its specific receptor (IL-23R) on Th17 cells to activate and maintain the clone, as well as to produce IL-17 and RANKL. Additionally, IL-17 can activate fibroblasts and macrophages that express RANKL which activates the osteoclast precursors to initiate joint and aloveolar bone erosion (4, 5).

It is noteworthy that the receptor of IL-23 can be released in soluble form (IL-23Rs) by several mechanics, already sIL-23Rs form a complex with IL-23 acting as inhibitor or activator of Th17. IL-17RA can be released too in soluble form (IL-17RAs) by alternative splicing and an form a complex with IL-17 acting as inhibitor (6, 7).

The IL-23/IL-17 axis in rheumatoid arthritis has also been studied and it was determined that IL-17A levels in serum were found higher in patients with RA compared to healthy subjects. Likewise, but in GCF of patients with RA and CP, was found higher IL-17 levels. Regarding the soluble receptor of IL-23, higher levels were reported in PC and PA compared to healthy subjects by our working group (8 – 13).

Currently, there are studies about the axis IL-23/L-17 in various types of samples, however, no reported studies of this axis in plasma cytokines and soluble receptors in patients with rheumatoid arthritis who have Chronic periodontitis.

Materials and Methods

Study subjects

The protocol was approved by the Research, Bioethics and Biosafety Committees of the University of Guadalajara. The study subjects were recruited to participate of Periodontal Clinic of the Dentistry School of University of Guadalajara and the Civil Hospital "Fray Antonio Alcalde". The purpose of this study was explained to each subject before he/she agreed to participate in the study, and their informed consent was obtained according to the Declaration of Helsinki and all subjects gave their written approval before participating based on the General Health Law and the NOM-008-SSA2-1993 norm.

Study groups

Medical and dental records were obtained for all participants and were diagnosed according to the classification of the American Academy of Periodontology 1999 (2) and by the Classification Criteria of the American College of Rheumatology prepared in 1987 (14).

Healthy subjects group (HS): n = 8, (8 females) mean age (37 ± 9)

Chronic periodontitis group (CP): n = 8 (8 females) mean age (45 ± 12)

Rheumatoid Arthritis group (RA): n = 8 (8 females) mean age (47 ± 12)

Rheumatoid Arthritis with Chronic Periodontitis group (RACP): n = 8 (7 females and one male) mean age (48 ± 10)

Sample collecttion

Blood obtained by venipuncture and was centrifuged to isolate plasma, which was immediately stored at -80°C until the cytokine ELISA for IL-23, IL-23R, IL-17 and IL-17RA was carried out with Enzyme –linked immunosorbent assay (ELISA)

Aliquots from either plasma or cytokine standars were added in triplicate to the wells of microtiter plates to determine the concentration of human IL-23, IL-23R, IL-17 and IL-17RA using Duo set ELISA kit (R&D Systems, Minneapolis, MN). The absorbance was read at 450 nm in a microplate spectrophotometer.

For the statistical analysis, the SPSS v 22.0 package for Windows was used, applying a Kruskal Whalis test for the comparison of independent means of three or more groups and significant differences were observed. Later, according to the Shapiro-Wilks test, the results showed an abnormal distribution, so a nonparametric Mann-Whitney U test was applied to know the differences between the means of SS, PC, AR and ARPC and a $P < 0.05$ It was considered significant.

Preliminary results

The results are presented as the mean ± the standard error of the levels of IL-23, IL-17 IL-23R and IL-17RA (pg/mL) and are represented in graphs.

IL-23 levels in plasma

IL-23 levels were assessed in plasma of four study groups mentioned where the highest levels were found in the RA group (1416.02 ± 366.04) compared to the group of SS [(288.0 ± 56.5) with the group of PC (309.4 ± 47.3) and with the ARPC group (458.95 ± 151.68) (Figure 1a).

IL-17 levels in plasma

The cytokine IL-17 in plasma was found higher in the group of rheumatoid arthritis (19.29 ± 5.94) compared to healthy subjects (3.48 ± 0.98), PC (10.38 ± 4.65) and AR & PC (1.88 ± 0.55) being the group of ARPC presented the lowest concentrations (Figure 1b).

IL-23Rs levels in plasma

IL-23Rs in healthy subjects was (16764.24 ± 2643.58) in PC group (20093.9 ± 5058.42), AR (24354.43 ± 1712.30) and ARPC (19489.91 ± 2358.58) being the RA group with higher levels and that of healthy subjects with the lowest levels (Figure 1c).

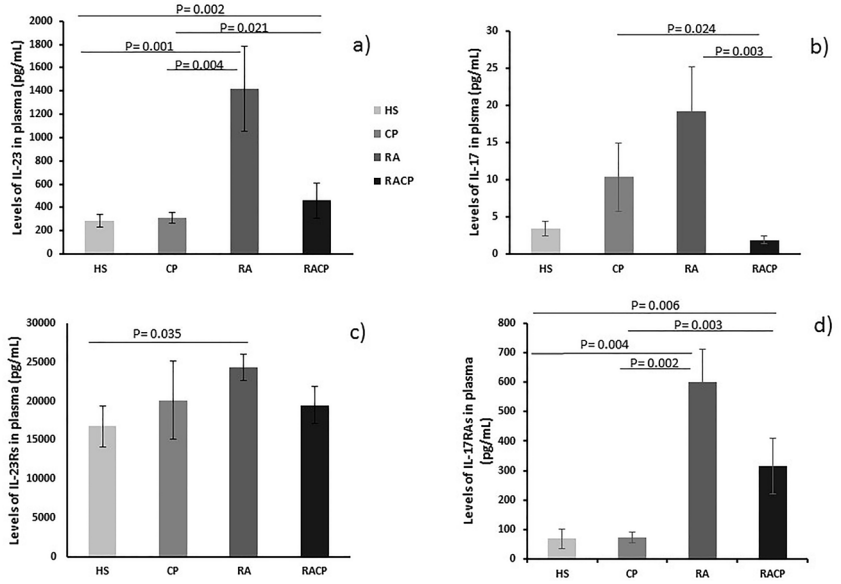


FIGURE 1: Levels of IL-23, IL-23R, IL-17A and IL-17RA in plasma from patients with Rheumatoid arthritis with Chronic periodontitis

IL-17RAs levels in plasma

IL-17RAs in plasma was found higher in the rheumatoid arthritis group (599.39 ± 112.52) compared to (HS) (68.9 ± 32.3), PC (73.8 ± 18.8) ARPC (314.53 ± 94.20) being the healthy subjects the group that presented the lowest concentrations (Figure 1d).

Conclusion: The levels of cytokines and soluble receptors analyzed were generally higher in the RA group compared with HS, CP and RACP. On the other hand, the group of healthy subjects presented the lowest levels of IL-23, IL -23Rs and IL-17 in plasma.

REFERENCES

1. Payne, J. B., Golub, L. M., Thiele, G. M., & Mikuls, T. R. (2015). The Link Between Periodontitis and Rheumatoid Arthritis: A Periodontist's Perspective. Curr Oral Health Rep, 2, 20-29.
2. Armitage, G. C. (1999). Development of a classification system for periodontal diseases and conditions. Ann Periodontol, 4(1), 1-6.

3. Brusca, S. B., Abramson, S. B., & Scher, J. U. (2014). Microbiome and mucosal inflammation as extra-articular triggers for rheumatoid arthritis and autoimmunity. Curr Opin Rheumatol, 26(1), 101-107.
4. Hajishengallis, G. (2014). Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. Trends Immunol, 35(1), 3-11
5. Lubberts, E. (2015). The IL-23-IL-17 axis in inflammatory arthritis. Nat Rev Rheumatol, 11(7), 415-429.
6. Edwards, D. R., Handsley, M. M., & Pennington, C. J. (2008). The ADAM metalloproteinases. Mol Aspects Med, 29(5), 258-289.
7. Franke, M., Schroder, J., Monhasery, N., Ackfeld, T., Hummel, T. M., Rabe, B., . . . Scheller, J. (2016). Human and Murine Interleukin 23 Receptors Are Novel Substrates for A Disintegrin and Metalloproteases ADAM10 and ADAM17. [research support, non-u.s. gov't]. J Biol Chem, 291(20), 10551-10561.
8. Ruiz Gutierrez, A. d. C., Herrera Mora, M. d. l. C., Zmora Perez, A. L., Melendez Ruiz, J. L., Martinez Rodriguez, V. d. C., & Guerrero Velazquez, C. (2014). Determinacion de los niveles de IL-17 en el liquido crevicular gingival de pacientes con periodontits cronica y agresiva. Revista Mexicana de Periodontologia, 46-50.
9. Moran, E. M., R. Mullan, et al. (2009). "Human rheumatoid arthritis tissue production of IL-17A drives matrix and cartilage degradation: synergy with tumour necrosis factor-alpha, Oncostatin M and response to biologic therapies." Arthritis Res Ther 11(4): R113.
10. Gumus, P., Buduneli, E., Biyikoglu, B., Aksu, K., Sarac, F., Nile, C., . . . Buduneli, N. (2013). Gingival crevicular fluid, serum levels of receptor activator of nuclear factor-kappaB ligand, osteoprotegerin, and interleukin-17 in patients with rheumatoid arthritis and osteoporosis and with periodontal disease. J Periodontol, 84(11), 1627-1637.
11. Rivadeneyra C., Rodriguez R., Ruiz A., Martinez V., Melenmdez J., Pita M., Guerrero C. (2017). Determinación de los niveles del receptor soluble de IL-23 en suero y plasma de pacientes con periodontitis cronica y agresiva. Revista Mexicana de Periodontología, 8(1), 5-10.
12. Himani, G. S., Prabhuji, M. L., & Karthikeyan, B. V. (2014). Gingival crevicular fluid and interleukin-23 concentration in systemically healthy subjects: their relationship in periodontal health and disease. J Periodontal Res, 49(2), 237-245.
13. van Baarsen, L. G., Lebre, M. C., van der Coelen, D., Aarrass, S., Tang, M. W., Ramwadhdoebe, T. H., Tak, P. P. (2014). Heterogeneous expression pattern of interleukin 17A (IL-17A), IL-17F and their receptors in synovium of rheumatoid arthritis, psoriatic arthritis and osteoarthritis: possible explanation for nonresponse to anti-IL-17 therapy? Arthritis Res Ther, 16(4), 426.
14. Aletaha, D., Neogi, T., Silman, A. J., Funovits, J., Felson, D. T., Bingham, C. O., 3rd, Hawker, G. (2010). 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum, 62(9), 2569-2581.

Expression of ADAM-10 Fas and Fas-L in NK and CD8+ T cells in peripheral blood of patients with chronic periodontitis

David Gómez Thomas[1], José Sergio Zepeda Nuño[1], Miriam Ruth Bueno[1], Elías Pérez Becerra[2], Elsy Verónica Palomares Castañeda[2], Vianeth M.C. Martínez Rodríguez[1], Ruth Rodríguez Montaño[1], Celia Guerrero Velázquez[1]

[1]Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
[2]Unidad de Patología Clínica, Guadalajara, Mexico
davidnemov@hotmail.com; patologiabucalgdl@gmail.com; ruthmyriamtop@hotmail.com; elias.perez@upc.com.mx; elssy_gy@hotmail.com; vianethperio@gmail.com; ruth_rodriguez21@outlook.com; celiagv2001@yahoo.com.mx

Keywords: Chronic Periodontitis, NK cells, Fas, FasL, T CD8+ cells, ADAM-10

Introduction: Periodontal disease is one of the most common chronic inflammatory diseases in humans. Clinical features of chronic periodontitis (PC) include loss of tissue insertion, loss of alveolar bone, periodontal pockets, and inflammation of gingival tissue. The virulence factors of the periodontopathogenic bacteria and the immune response against them are involved in the destruction of the tissues observed in periodontitis. The immune cellular response plays a very important role against bacteria in periodontal diseases. CD8+ T lymphocytes and NK cells trigger the extrinsic pathway of apoptosis through the action of FasL and Fas, these cells express on their surface a disintegrin and metalloproteinase domain-containing protein 10 (ADAM-10), which through of FasL cleavage, it can modulate the apoptosis of target cells, however the correlation between the levels of these proteins in patients with PC is unknown.

Objective: To determine the expression of ADAM-10 Fas and FasL in NK cells and CD8+ T lymphocytes in patients with chronic periodontitis. Methods. We included 7 healthy control subjects and 10 patients with CP diagnosed according to the International Workshop 1999 and through flow cytology in peripheral blood, the percentages and mean fluorescence intensity (MFI) of ADAM10, Fas and FasL were determined.

Results: No significant differences were found in the percentages and MFI of Fas and ADAM-10 in NK and CD8+ cells between the group of healthy subjects and the group of patients with PC. However, differences were found in the percentages of FasL in

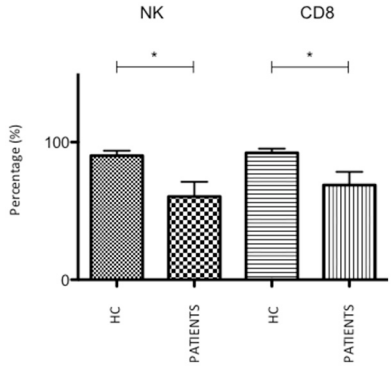


FIGURE 1: Percentage of expression of FasL in NK and CD8+ T cells. HC, healthy controls

NK and CD8+ T cells between healthy subjects and patients with PC ($p = 0.021$ and $p = 0.045$ respectively). (Figure 1)

Conclusions: There is an overexpression of FasL in cells of patients with CP, this imbalance could be an important regulator in the immune response against bacteria associated with implantation and development in PC.

Effect of Cymbopogon citratus and Artemisia mexicana on the mRNA expression of TNF-alpha and IFN-gamma

Omar Fernández-Rivera, Monserrat Sofia López Padilla, Teresita de Jesús Nolasco Pérez, MARTHA LEGORRETA-HERRERA

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
qfbfdz@gmail.com; alcume_100@hotmail.com; teresyta.qfb@gmail.com; marthal@unam.mx

Keywords: Malaria, tNF-alpha, Cymbopogon citratus, INF-gamma, Artemisia mexicana

Malaria is a protozoan infection responsible of 214 million cases and 438 thousand deaths in 2015. Immune response to Plasmodium infection is characterized by the production of pro-inflammatory cytokines and cellular immune responses that lead to inflammation. The complexity of the Plasmodium life cycle and multiple immune evasion strategies developed by Plasmodium is a challenge to malaria erradication. This parasite has developed drug resistance to practically all existent antimalarial drugs. This includes emerging resistance to artemisinin-based combination therapies.

Drugs derived from natural source, especially plants, represent a significant proportion of the pharmaceutical market, particularly in malaria therapy; examples include quinina from Chinchona sp. and artemisinin from Artemisia annua. Their advantage comes from the synergistic interactions of their components and their innate affinity to biological receptors. The pharmacological justification of these principles could provide the basis for development of plant-based traditional medicine as a reliable therapeutic tool. Nevertheless one hypothesis that has not been exploited in conventional antimalarial therapy is the synergistic interaction of antimalarial and anti-inflammatory effects coming from compounds present in plants. Mostafa A. et al, demonstrated that Artemisia annua dried leaves had a higher antimalarial effect compared to the equivalent artemisinin dose. Interestingly, the dried leaves treatment overcame the Plasmodium resistance to artemisinin.

Following the work of Mostafa et al, we used Cymbopogon citratus, and demonstrated the higher antimalarial efficacy of the whole C. citratus plant compared with the herbal infusion, however we did not determine the mechanism of action. Therefore, in this work we evaluated the activity of both C. citratus and Artemisia mexicana whole plants on the expression of pro-inflammatory cytokines TNF-alfa and IFN-gamma in the spleen of CBA/Ca mice infected with P. berghei ANKA.

Methods: Ten groups of 5 mice were daily treated starting 4 days before infection with either vehicle, chloroquine (10mg/Kg), Cymbopogon citratus (1600mg/Kg) or Artemisia annua (1600mg/Kg). Mice were infected with Plasmodium berghei ANKA. On day 8 post-infection all the groups of mice were sacrificed and spleens were removed to extract mRNA which was reverse-transcripted to cDNA and qPCR amplified to TNF-alfa and INF-gamma genes.

Results and Conclutions: A. mexicana down regulated the mRNA expression of both TNF-alpha and IFN-gamma. Interestingly, C. citratus only down regulated the mRNA expression of IFN-gamma. Since both cytokines are involved in both parasite elimination and pathology via increasing the inflammatory process, the fact that A. mexicana and C. citratus decreased both inflammatory cytokines preserving their antimalarial activity suggest their potential use as combined anti-malarial therapy.

Mercury enhanced virulence of Sporothrix schenckii by fungal exposition to mercury and Th1/Th17 cells response in mice

Damiana Téllez-Martínez[1], Alexander Batista Duharte[1], Deivys Leandro Portuondo Fuentes[1], Miriam SJB Lastre González[2], Oliver Pérez[2,3], Iracilda Zeppone Carlos[1]

[1]São Paulo State University (UNESP), School of Pharmaceutical Sciences, Araraquara, Brazil
[2]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[3]Escuela Latinoamericana de Medicina, La Habana, Cuba
damianatellezm@gmail.com; batistaduhartea@gmail.com; deivysleandro@gmail.com; oliver.perez@infomed.sld.cu; oliverperez@giron.sld.cu; carlosiz@fcfar.unesp.br

Keywords: Infection, Mercury, mucosal immunity, Sporothrix schenckii, Th1/Th17

Introduction: The impacts of environmental contamination on emergence or re-emergence of infectious diseases have become major concerns in environmental health. Mercury (Hg) is a global environmental contaminant of water and soils. A significant amount of Hg is released during artisanal goldmining in the world1. Sporotrichosis is an emergent chronic granulomatous mycotic disease caused by pathogenic species of Sporothrix schenckii, a microorganism that lives in soils from tropical and subtropical countries. S. schenckii develop opportunistic systemic infections in immunodeficient patients2. The influence of environmental contaminant on fungal virulence and sporotrichosis outbreaks have been hypothesized3,4. Species of S. schenckii has been isolated from soils highly contaminated with Hg and several outbreaks have been described in gold mining regions with high Hg contamination5. The average concentration of inorganic Hg in soil is in the range of 0.07 to 1.53 ppm (mg kg-1)6. Aim: To evaluate the influence of Hg in the virulence of S. schenckii and the post-infection Th1/Th17 immunopolarization.

Material and Methods

Microorganisms and Culture Conditions

S. schenckii ATCC 16345, originally obtained from a human case of diffuse lung infection (Baltimore, MD) and kindly provided by the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) was used for all experiments. For mice infection and heat-killed S. schenckii yeast (HKss) preparation, a piece of the fungal mycelium grown on mycosel

agar tubes was transferred to an Erlenmeyer flask with 100 mL of Sabouraud broth containing either 1 or 10 ppm of HgCl2. The flasks were cultured for 7 days at 37°C to achieve the mycelium-to yeast conversion. Then, cultures were filtered through sterile gauze and the yeast was adjusted to 1x106/mL in phosphate buffer solution (PBS).

Animals and Experimental Design

Male Swiss mice, 5–7 weeks old at the time of inoculation, were obtained from the Animal House at the School of Pharmaceutical Sciences, UNESP (Araraquara, SP, Brazil). Mice were housed in micro isolators under controlled conditions. Seven animals by groups were intraperitoneally inoculated with 106. S. schenckii yeast cells (untreated or previously cultured with 1 or 10 ppm of HgCl2 in PBS), or with an equal volume of PBS alone (Control). Assessment of the systemic fungal load was performed by counting the colony forming units (CFU) grown on Mycosel agar plates after the spread-plating of a previously determined dilution of the spleen and liver macerates7. All animal procedures were performed according to the guidelines of the Brazilian College of Animal Experimentation (COBEA).

Flow Cytometry

The following anti-mouse mAb for phenotypic evaluation of Th1/Th17 cells were used: anti-CD16/CD32 purified; anti-CD3 FITC; anti-CD4 APC; anti-IL-17 PE; and anti-IFNγ PerCP-cy5.5 all from eBioscience. Splenocytes were stimulated in vitro with Cell Stimulation Cocktail containing phorbol 12-myristate 13-acetate (PMA)/ionomycin for cell stimulation and Brefeldin A/Monensin for intracellular retention of the induced cytokines. Splenocytes were assessed for the frequency of Th1 (IFNγ single-positive Th cells), Th17 (IL-17 single-positive Th cells), Th1/Th17 (IFNγ+IL-17+ Th cells).

Measurement of the ex vivo Release of Cytokines

Splenocytes from infected and control mice were cultured in RPMI complete medium for 24 h at 37°C and 5% CO_2 on flat bottom 48-well tissue culture plates in the presence of HKss, at a splenocyte:yeast ratio of 1:5. Final concentrations were 2.5×106 splenocytes/mL and 1.25×107 yeast cells/mL. Concanavalin A (0.25 g/mL) and RPMI alone were used as positive and negative controls, respectively. Cytokine concentrations (IFNγ, IL-17 and IL-4) in cell-free supernatants were measured by specific ELISA according to the manufacturer's instructions (eBioscience).

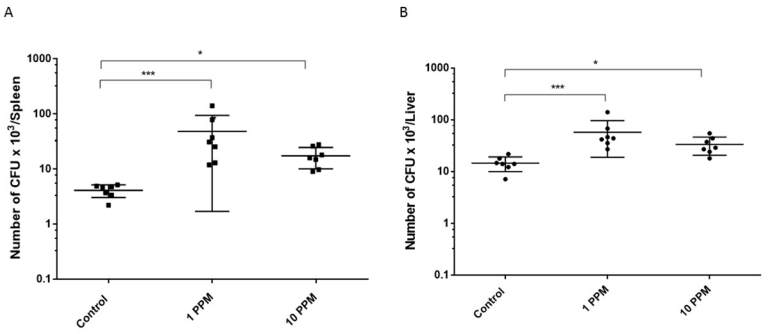


FIGURE 1: Fungal burden at 7th day in spleen and liver of mice infected with S. schenckii yeast previously cultured with either 1 or 10 PPM of HgCl2. * ($p < 0.05$), *** ($p < 0.001$)

Statistical Analysis

Statistical analysis was performed in GraphPad Prism ver. 6.01, by non-parametric Kruskal-Wallis test with Dunn multiple comparisons test. Confidence interval was set at 95% for all tests.

Results: Fungal burden in spleen and liver. The fungal load in the spleen and liver was evaluated at 7th day post-infection as an expression of systemic dissemination and virulence (Fig. 1). Those mice infected with previously treated fungi with either 1 or 10 ppm of HgCl2, exhibited a higher fungal burden in spleen and liver than those infected with non-treated fungi ($p < 0.05$). However, the stronger significance was noted for 1 ppm ($p < 0.001$).

Th1/Th17 Response

Mice infected with S. schenckii yeast previously treated with 1 ppm of HgCl2 induced a stronger response of IFNγ+IL17+ lymphocytes in the CD3+CD4+, population at 7th day of infection in comparison with non-treated yeast and those treated with 10 ppm (Fig. 2). Similarly, a highest concentration of IFNγ and IL-17A was also detected in the supernatant of splenocytes cultures of this group of mice after in vitro stimulation with Hkss.

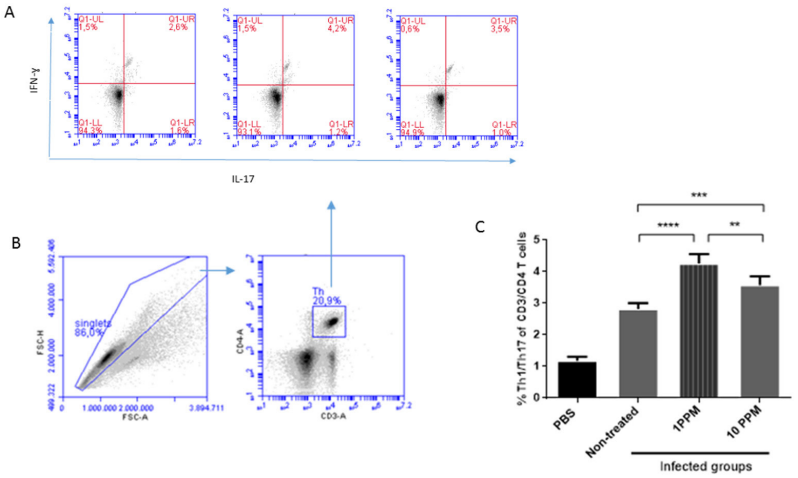


FIGURE 2: Changes in spleen's Th1/Th17 frequency at 7th day in mice following intraperitoneal infection with S. schenckii yeast previously cultured with either 1 or 10 PPM of HgCl2. Representative plot (A) and (B) gating strategy for determination ofTh1/Th17 (intracellular cytokines) responses induced at 7th days in male Swiss mice infected with S. schenckii yeast previously cultured with either 1 or 10 PPM of HgCl2 (C). ** ($p < 0.01$); ***($p < 0.001$); ****($p < 0.0001$)

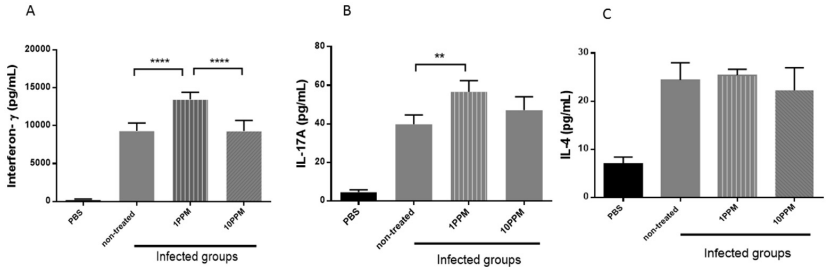


FIGURE 3: Cytokines profile induced at 7th day post-infection in mice infected with S. schenckii yeast previously cultured with either 1 or 10 PPM of HgCl2. Splenocytes from infected or control mice were activated in vitro with HKss. A) IFNγ; B) IL-17A; B) IL-4. **

Non-relevant production of IL-4 was detected in any case (Fig. 3). These results suggest that the higher dissemination of yeast treated with 1 ppm of $HgCl_2$, also stimulated a stronger Th1/Th7 response, two determinant ways for S. schenckii clearance7.

Conclusions: These results suggest that environmental exposition of S. schenckii to 1 and 10 ppm of $HgCl_2$ enhance their virulence and it capacity to stimulate Th1/Th17 responses in the host. Exposition to 1 ppm induced stronger effect on S. schenckii virulence than 10 ppm. More studies are necessary to evaluate the relevance of this finding on outbreaks sporotrichosis in contaminated regions with this heavy metal.

Determination of inflammation, translocation and activation biomarkers in serum of elderly HIV+ patients

Sarah Ratkovich-Gonzalez, Karina Sanchez-Reyes, Judith Carolina De Arcos Jimenez, Mariana del Rocio Ruiz-Briseño, Maria Elizabeth Preciado-Ortiz, Monserrat Alvarez-Zavala, Luz A Gonzalez-Hernandez, Jaime Andrade-Villanueva, Moises Ramos-Solano

Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
sarahqfb@gmail.com; karina_reyesqfb@hotmail.com; judcath@gmail.com; lilieth@live.com.mx; elizabeth.nuta@gmail.com; monse_belan@hotmail.com; luceroga08@gmail.com; drjandradev@gmail.com; biologmoises@gmail.com

Keywords: Chemokines, Cytokines, HIV, Inflammation, Elderly, LPS, Hs-CRP, sCD14, Inmunosenescence, IFABP

Background: Aging is a complex process with negative effects on a wide variety of physiological processes. Aging process attenuates host ability to mount an efficient immune response, this may contribute to the increased incidence and severity of infectious diseases in elderly people. Immunosenescence refers to the gradual deterioration of the immune system in positive correlation to age.

The hallmarks of HIV infection are a constant and progressive T CD4+ cells depletion and immune activation. Currently, antiretroviral therapy prolongs life expectancy in HIV patients however, those patients who have been in treatment for an extensive period of time, have a high risk of mobilities related with aging as cardiovascular disease, cancer, and other illnesses, this susceptibility is consistent with the changes in the immune system seen in elderly patients and are likely related to persistent inflammation. The elevation of plasmatic markers of inflammation has been associated to the development of comorbidities non-related to AIDS. Immune activation in HIV infection is characterized by the elevated concentration of biomarkers such as proinflammatory cytokines; C-reactive protein (CRP), which is considered a predictor of HIV disease progression; sCD14 a marker of monocyte response to LPS who is an independent predictor of mortality in HIV infection and intestinal fatty acid-binding protein (I-FABP), a marker of damage and inflammation to the small intestinal mucosa.

Objective: Describe inflammation, translocation and immune activation biomarkers and correlate those with the T CD4+ absolute count in elderly HIV+ patients.

Methodology: With a previous informed consent, the HIV+ patients were recruited from the HIV clinic at Hospital Civil Fray Antonio Alcalde in Guadalajara, Mexico, under the following criteria: elderly (above 50 years old) HIV+ patients, with more than two years of stable Antiretroviral Therapy, in virological control, a CD4+ absolute count of >200 cel//μL, and with a stable body weight.

A blood sample was taken from patients, after that the serum was separated and stored at -80ºC until used. The inflammatory cytokines (IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33) were measured by flow Cytometry using the LEGENDplex inflammation panel, the sample were processed following the manufacturer's instructions and the results were analyzed using the LEGENDplex™ Data Analysis Software. The translocation biomarkers IFABP and LPS as well as the soluble proteins sCD14 and hs-CRP were performed by ELISA following the manufacturer's instructions; the T CD4+ count was evaluated by flow cytometry using FACSCalibur System.

Results: The age mean was 57 ± 5 years, the gender distribution was 16 males and 4 females, nadir CD4+ T cells median was 74 (IQR: 39,220) cells/μL, and the current (at the moment of the study) CD4+ T absolute count median was 409 (IQR: 279, 744) cells/μL.

In the case of cytokines, the mean obtained for each cytokine was: for IL1β 14.61 ± 5.54, IFNα 23.85 ± 18.7, IFNγ 811.95 ± 729.26, TNFα 17.29 ± 7.56, MCP1 734.39 ± 407, IL-6 74.57 ± 20.29, IL-8 15.73 ± 18.77, IL-10 11.36 ± 6.75, IL-12 9.45 ± 4.38, IL-17 47.79 ± 52.35, IL-18 579.1 ± 424.32, IL-23 34.8 ± 25.1 and IL-33 91.75 ± 82.88 (pg/mL); respectively.

Translocation Biomarkers

IFABP had a mean of 5.08 ± 4.44 ng/mL and LPS 53.95 ± 30.45 pg/mL. Soluble proteins: CD14s had a mean of 9.47 ± 3.45 ng/mL and hs-CRP 3.5 ± 2.18 ng/mL. None of the correlations between the biomarkers and CD4+ absolute count was significative, however we found a strong positive correlation between CD14s and hs-RCP.

Conclusion: Our results point to a strong inflammatory profile in these individuals, denoted by the high levels of proinflammatory cytokines and chemokines, as well as the presence of the hsCRP whose existence in serum is a marker of inflammation and has been used in HIV patients as an indicator of the disease progression.

Oxidative stress induced by glycolysis drives the crosstalk between monocytes and T cells in human Chagas disease

Liliana Maria Sanmarco[1,2], Natalia Eberhardt[2], Luz Piedad Quebrada Palacio[3], Pamela Victoria Martino Adami[2,4], Laura Marina Visconti[5], Angel Ramón Minguez[5], Laura Morelli[2,4], Miriam Postan[3], Maria Pilar Aoki[1,2]

[1]Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[3]Instituto Nacional de Parasitología Dr. Mario Fatala Chabén, Buenos Aires, Argentina
[4]Leloir Institute Foundation (FIL), Buenos Aires, Argentina
[5]Hospital Nuestra Señora de la Misericordia, Córdoba, Argentina
lili-sanmarco@hotmail.com; neberhardt@fcq.unc.edu.ar; lupi90@gmail.com; pmadami@leloir.org.ar; lau_visconti81@yahoo.com.ar; angelminguez04@yahoo.com.ar; lmorelli@leloir.org.ar; miriampostan@yahoo.com; paoki@fcq.unc.edu.ar

Keywords: Nitric Oxide, tyrosine nitration, classical monocytes, Trypanosoma cruzi infection, Non classical monocytes

Chagas disease is caused by Trypanosoma cruzi infection and constitutes a major public health problem in Latin America due to its prevalence, morbidity and mortality. The host's ability to control infection is substantial, but not fully effective, since most infected individuals tightly limit parasite load but fail to completely clear the infection due to diverse and fascinating immune evasion processes. Although nitric oxide (NO) is key as anti-trypanosomal agent, persistent levels of NO are also involved in the induction of lymphocyte unresponsiveness, since it triggers the nitration of T-cell surface proteins. In this sense, we have recently reported that circulating leukocytes from Chagas disease patients exhibit increased NO production concomitant with augmented nitration of CD8+ T cells and impaired cytotoxic functions. In addition, a substantial contraction of T cell compartment at the expense of CD8+ T cells is observed in peripheral blood from Chagas disease patients. Taking into account that monocytes (Mo) are one of the main sources of NO; our aim was to elucidate the crosstalk between Mo and T cells in human Chagas disease, characterizing the frequency of Mo subsets and their effector functions, and determining the mechanism that drives Mo-T cell interaction. Similar to bacterial and viral infections, the proportion of circulating classical Mo decreased ($p<0.0001$), while the frequency of non-classical subset increased ($p<0.01$) in seropositive patients compared to seronegative donors. However, in control donors and patients the percentage of non-classical Mo positive for NO and reactive oxygen species (ROS) production were higher than the frequency of NO+ and ROS+

classical Mo, and the proportion of non-classical Mo producing IL-10 and IL-6 were lower than the frequency of IL-10+/IL-6+ classical Mo. Mammalian cells adapt to inflammatory or infectious conditions by activating hypoxia-inducible factor (HIF)-1α, a master regulator of transcription. HIF increases expression of genes that trigger vascularization, vasodilation (iNOS), erythropoiesis and glycolysis. Indeed, Mo from seropositive individuals showed higher expression of HIF-1α ($p<0.01$) while the frequency of IL-1β ($p<0.001$), IL-6 ($p<0.05$), IL-10 ($p<0.01$) and NO ($p<0.05$) producing Mo is also higher compared to control donors. Nevertheless, Mo from both groups produced similar levels of ROS. Strikingly, plasma levels of IL-6, IFN-γ, TNF and IL-4 were similar in seronegative and seropositive individuals. Interestingly, although IL-1β levels were higher, the amount of IL-10 was lower in plasma from Chagas disease patients compared to control donors. To evaluate the effect of glycolysis on the Mo-T cell crosstalk upon infection, we treated peripheral blood mononuclear cells with a synthetic glucose analog 2-deoxyglucose (2-DG) that inhibits glycolysis, and then infected them with T. cruzi trypomastigotes. We found that 2-DG increased mannose receptor (CD206) expression on Mo/macrophages (Ma), decreasing the production of IL-6 and the levels of NO in culture supernatants as well as the frequency of IL-1β- and NO-producing Mo/Ma. Moreover, glycolysis inhibition decreased the frequency of infection-induced T cell nitration. Considering that CD8+ T cells have become a focus of intense studies for the development of vaccines against intracellular parasites, our findings could have key clinical relevance in this research field.

Trypanosoma cruzi infection induces first Wnt/β-catenin and then Wnt/Ca+2 pathway activation in macrophages which favour the parasite replication

Ximena Volpini[1,2], Laura Fernanda Ambrosio[1,2], Laura Fozzatti[1,2], Constanza Insfrán[1,2], Claudia Cristina Motran[1,2]

[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI CONICET), Cordoba, Argentina
[2]Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina
xvolpini@fcq.unc.edu.ar; lambrosio@fcq.unc.edu.ar; lfozzatti@fcq.unc.edu.ar; cinsfran@fcq.unc.edu.ar; cmotran@fcq.unc.edu.ar

Keywords: Calcium, Chagas Disease, Infection Control, Wnt Proteins, beta-Catenin

Chagas' disease, caused by the protozoan parasite Trypanosoma cruzi, represents a major cause of heart disease and cardiovascular-related deaths in endemic areas, and causes a significant economic burden on the affected countries. Approximately 8 million people are infected with T. cruzi in Central and South America, and at least 120 million are at risk of infection. Currently, there are no vaccines available to prevent Chagas disease and treatment options are limited to anti-parasitic drugs that are expensive, not well tolerated, and effective only during short periods of the acute phase.

During the infection with T. cruzi, both a strong inflammatory and an efficient regulatory response are essential to restrict parasite replication and prevent immunopathology. At the acute phase of infection, macrophages can act as "host" cells for the parasites and, in turn, as effector cells in the early anti-parasitic immune response. In these cells, parasite replication can be either inhibited or favoured leading to dissemination to other sites within the body. Thus, the targeting of specific signaling pathways could modulate macrophage response to restrict parasite replication and instruct an appropriate adaptive response.

Recently, it has become apparent that Wnt signaling pathway exerts immunomodulatory functions during inflammation and infection. We have tested the hypothesis that during T. cruzi infection, the activation of Wnt signaling pathway in macrophages plays a role in modulating the inflammatory/tolerogenic response and therefore regulating the control of parasite replication. We have observed that early after T. cruzi infection of bone marrow derived macrophages (BMM), β-catenin was activated and Wnt3a, Wnt5a, and some Frizzed receptors as well as target genes of Wnt/b-catenin pathway were up-regulated, with Wnt proteins signaling sustaining the activation of Wnt/²-catenin pathway and then activating the Wnt/Ca+2 pathway. Wnt signaling pathway activation

was critical to sustain the parasites replication in BMM; since the treatments with specific inhibitors of ²-catenin transcriptional activation or Wnt proteins secretion limited the parasite replication. Mechanistically, inhibition of Wnt signaling pathway armed BMM to fight against T. cruzi by inducing the production of pro-inflammatory cytokines and IDO activity and by down-regulating arginase activity. Likewise, in vivo pharmacological inhibition of the Wnts' interaction with its receptors controlled the parasite replication and improved the survival of lethally infected mice.

It is well established that T. cruzi infection activates a plethora of signaling pathways that ultimately regulate immune mediators to determine the modulation of a defined set of effector functions in macrophages. In the present study we reported a new signaling pathway that is activated by the interaction between T. cruzi and host innate immunity, establishing a new conceptual framework for the development of new therapies.

Klebsiella pneumoniae prevents the release of neutrophil extracellular traps by human neutrophils

Federico Birnberg Weiss, Luis A. Castillo, Nahuel Rodriguez Rodrigues, Daiana Martire Greco, Verónica I. Landoni, Gabriela C. Fernández

Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina

federicobirnberg@hotmail.com; luiscastillo718@gmail.com; nahuelrodriguezr@gmail.com; daianamartire@hotmail.com; vilandoni@yahoo.com.ar; gfernandez@hematologia.anm.edu.ar

Keywords: Klebsiella pneumoniae, Neutrophils, NEtosis, Carbapenem resistance, Evasion mechanism

Bacterial infections remain an important cause of morbidity and mortality worldwide, especially in immune-compromised patients. Although much progress has been made in its treatment, the appearance of new strains with acquired multiple antibiotic resistances and the growing number of immunosuppressed patients represents great therapeutic challenges.

Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria are a group of emerging highly drug-resistant Gram-negative bacilli causing infections associated with significant mortality, mainly associated to carbapenem resistance, one of last option of antimicrobial treatment.

Neutrophil (PMN)-mediated response is essential for host to first combat bacterial infection. However, previous results showed that Klebsiella pneumoniae (Kpn) may be resistant to PMN bactericidal mechanism such as phagocytosis.

The release of neutrophil extracellular traps (NET's) is a major immune mechanism intended to capture and destroy pathogens. These histone- and protease-coated DNA structures are released by PMN in response to a variety of stimuli and have been identified in the airways of patients with respiratory infection, cystic fibrosis, peritonitis, acute lung injury, chronic obstructive pulmonary disease and septicemia. Some bacteria such as Staphylococcus aureus, Yersinia enterocolitica, Yersinia pseudotuberculosis and E. coli have been reported to induce NETosis.

Since the discovery of NET's over a decade ago, evidence that "NET evasion" might act as an immune protection strategy for bacterial pathogens, including group "A" Streptococcus, Bordetella pertussis, and Haemophilus influenzae, has been growing, with the majority of these studies being published in the past 2 years.

Hence, since the evasion of NET's appears to be a widespread strategy to allow pathogen proliferation and dissemination, and is currently a topic of intense research interest, the aim of this study was to investigate whether Kpn was able to modulate the bactericidal response of PMN, focusing on NET's formation. For this purpose, we determine NETosis on human purified PMN in response to three different strains, two Kpn, the KPC producer strain (Kpn-KPC) and the non carabapenem resistant Kpn ATCC 700603 strain (Kpn-ATCC), and other opportunistic enterobacteria such as Escherichia coli (E. coli) ATCC 25922.

PMN-bacteria were incubated for 3 hours, NET's released was observed by confocal microscopy and the area was measured by the Image J® software, after DNA and Elastase staining. In addition, PMN-released Myeloperoxidase (MPO) and DNA were measured in supernatants of PMN-bacteria co-cultures.

We first found that PMN failed to produce NETosis when were challenged with Kpn-KPC or Kpn-ATCC strains, in spite to different PMN:Kpn ratios (1:10 and 1:1) as determined by both confocal microscopy and measure of NET's released, while E. coli was a potent NET's inducer, compared to unstimulated PMN (Ctrl) (NET's area, µM2 = Ctrl: 66.5±12, Kpn-KPC: 95.7±16, Kpn-ATCC: 174±43, E. coli: 21754.4±2289; Released DNA, ng/mL = Ctrl: 234.8±29, Kpn-KPC: 195.4±37, Kpn-ATCC: 160±26, E. coli: 930±52; Released MPO, O.D = Ctrl: 0.05±0.01, Kpn-KPC: 0.08±0.004, Kpn-ATCC: 0.08±0.005, E. coli: 0.37±0.03, $p < 0.05$ E. coli vs all others).

This data was in accordance with PMN-bacterial killing determined by colony forming units (CFU) quantification after 3 hours of PMN-bacteria co-cultures. In this sense, the percentage of bacterial survival was significantly higher for Kpn-KPC and Kpn-ATCC compared to E. coli ($p < 0.05$).

Previous results from our laboratory have shown an inability of Kpn to induce a respiratory burst in PMN. Considering this result and the fact that intracellularly produced reactive oxygen species are necessary for NET's formation, we decided to investigate if the failure in NET's formation was related to the absence of one of this reactive oxygen species. Constant supply of peroxide (H_2O_2) can be guarantied by glucose oxidase (GO) addition in a culture medium with glucose as GO substrate. Therefore, PMN were incubated for 3 hours in the presence of Kpn-KPC and GO and NET's were evaluated after the incubation period. In spite of constant reactive oxygen species supply

by GO, Kpn-KPC still failed to induce NETosis; moreover, the presence of Kpn-KPC inhibited the intrinsic capacity of GO to induce NETosis through the production of peroxide (Released DNA, ng/mL = GO: 496±29, Kpn-KPC: 126±17, Kpn-KPC+GO: 180±21, $p<0.05$ Kpn-KPC+GO vs GO), indicating that the deficiency in reactive oxygen species generation by PMN was not related to NETosis evasion of Kpn-KPC.

Finally, in order to determine if the lack of NET's induction of Kpn-KPC could prevail in the presence of other NET's inducing bacteria, we evaluated NETosis of PMN in mixed E. coli+Kpn-KPC cultures. Results from confocal microscopy and released NETs analysis revealed that the presence of Kpn-KPC reduced E. coli-induced NETosis (NET's area, μM2 = E. coli: 18325.4±1851, E. coli+Kpn-KPC: 8091.2±862; Released DNA, ng/mL = E. coli: 821±26, E. coli+Kpn-KPC: 547±16; Released MPO, O.D = E. coli: 0,947±0.03, E. coli+Kpn-KPC: 0.608±0.02; $p<0.05$ E. coli vs E. coli+Kpn-KPC).

In summary, our results indicate that Kpn is able to subvert one of the most relevant bactericidal mechanisms of PMN, NET's formation, and this can be related to a higher survival of Kpn compared to other bacteria.

Analysis of the polymorphism 1729+55del4 in SLC11A1 gene and its association with the development of cutaneous leshmaniasis in Campeche, México

Noe Alejandro Laredo[1], Alejandra Del Carmen Ramos Varguez[1], Jorge Antonio Canto Herrera[1], José Alberto Diaz-Quiñonez[2], Juan Carlos Carpio Pedroza[2], Octavio Cesar Rivera Hernandez[2], Elsy Beatriz Canche Pool[1]

[1]Autonomous University of Yucatan, Mérida, Mexico
[2]Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), México, Mexico
alejandro_laredo1992@hotmail.com; alheeramos@hotmail.com; cherrera@correo.uady.mx; alberto.diaz@salud.gob.mx; juan.carpio@salud.gob.mx; octavio.rivera@salud.gob.mx; elsy.canche@correo.uady.mx

Keywords: Leishmaniasis, susceptibility, SLC11A1, Subclinical infection, clinical infection

Leishmaniasis is caused by intracellular protozoan parasites of the genus Leishmania, whose spectrum of clinical manifestations include localized cutaneous, diffuse cutaneous, mucocutaneous, and visceral disease. [1]

In Mexico, the sylvatic region in Campeche and Quintana Roo, are highly endemic areas of cutaneous leishmaniasis caused by L. (L.) mexicana. In those areas, the existence of clinical and subclinical infection has been documented.

The clinical infection develops as an ulcerated lesion in the area of sandfly bite. It is chronic, disabling, and usually does not resolve spontaneously unless the treatment is administered. [2] Instead, people with subclinical infection do not show signs and symptoms of the disease but they are positive to the Leishmanin test. [3]However, the factors that contribute to the development clinical or subclinical infection are unknown.

In this regard, the polymorphisms in SLC11A1 (solute carrier family 11 member 1 protein), formely NRAMP1 (Natural Resistance Associated Macrophage Protein 1) gene, has been studied for its role in susceptibility to develop infectious diseases such as tuberculosis, leprosy, meningococcal meningitis, visceral leishmaniasis, and HIV infection, as well as to autoinmune diseases such as rheumatoid arthritis, diabetes, sarcoidosis, inflammatory bowel disease and Kawasaki disease. [4]

Specifically, the deletion in the 3UTR region (1729 + 55del4) has been associated with susceptibility to development tuberculosis and cutaneous leishmaniasis by L. (V.) braziliensis. [5] Therefore in this work, we examined this polymorphism in clinical and subclinical infection caused by L. (L.) mexicana.

We performed the study with blood samples collected from an endemic area of cutaneous leishmaniasis in the state of Campeche Mexico. DNA was obtained and Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms (PCR-RFLP) was used for the genotiping.

Preliminary results indicate a homozygous genotype (del/del) in clinical infection and homozygous genotype (TGTG/TGTG) in subclinical infection, but is necesary to increaze the sample size to determine an association.

REFERENCES

1. World Health Organization. Control of Leishmaniasis. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. Geneva: WHO; 2010. WHO Technical Report Series:949.
2. Andrade Narváez FJ, García Miss MR, Cruz Ruiz AL, Canto Lara SB, Simmonds Díaz E, Estudio preliminar de correlación Clínica, histopatológica e inmunológica de la leishmaniosis cutánea mexicana en el hombre. 1984 Arch Invest Med Méx;15:267-280.
3. González A, Biagi F, Asymptomatic infections in Mexican Cutaneous Leishmaniasis. 1968 Dermatol Int;7(1):8-9.
4. Donninger H, Cashmore T, Scriba T, Petersen D, Janse V, Hayes V. Functional analysis of novel SLC11A1(NRAMP1) promoter variants in susceptibility to HIV. 2004 J Med Genet; 41(4):1-3.
5. Castellucci L, Jamieson SE, Miller EN, Menezes E, Oliveira J, Magalhães A, Guimarães LE, Lessa M, Ribeiro de Jesus A, Carvalho EM, Blackwell JM, CXCR1 and SLC11A1 polymorphisms affect susceptibility to cutaneous leishmaniasis in Brazil: a case-control and family-based study. 2010 BMC Medical Genetics; 11(10):1-8

Immune response of human cerebral endothelial cells to zika virus

Alejandro Ortiz

Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
alexanderos207@gmail.com

Keywords: Blood-Brain Barrier, Guillain-Barre Syndrome, Neuropathology, Immuno Response, Zika virus infection

Alejandro Ortiz-Serrano1, Jesús Miguel Torres-Flores1, Manuel Alejandro Mosso-Pani1, 2, Ma. Isabel Salazar-Sánchez1*
*1Laboratorio de Virología e Inmunovirología. Departamento de Microbiología. Escuela Nacional de Ciencias Biológicas –IPN. 2Hospital General Regional 1 "Carlos McGregor Sánchez Navarro" Instituto Mexicano del Seguro Social, Mexico city, Mexico. *alexanderos207@gmail.com*

Introduction: Zika virus (ZIKV) significant damages cerebral level in humans, these include microcephaly in fetuses and neuropathies in adults, still, the molecular mechanisms underlying these events are not fully understood. Increasing evidence supports the fact that a significant dysfunction in the blood-brain barrier (BBB) occurs during infection, surprising in adult patients too. The loss of integrity of the BBB might be determined by damage at the tight junction's level produced by ZIKV infection [1–5].

Objective: To evaluate the effect of Zika virus infection and the immune response of endothelial cells of the cerebral microvasculature.

Materials and Methods: To determine whether the infection with ZIKV affects the properties of permeability of cerebral microvasculature endothelial cells (CMEC) known as HBEC-5i, in vitro, we evaluated their permissibility to ZIKV infection by Transwell® assay. Proinflammatory cytokine was quantified in supernatant of CMEC at different times during ZIKV infection of CMEC by Citometric Bead Array BD™ assay. Finally, the occludin protein expression in CMEC exposed to ZIKV was evaluated by indirect immunofluorescence in a confocal microscope and western blot.

Results: We found that endothelial cells of the HBEC-5i line were able to infect with ZIKV and replicate it. We assessed production of TNF-α in the supernatant of the CMEC infected with ZIKV. Also, we analyzed changes in occludin localization during a time course of infection with ZIKV.

Our findings suggest that alterations in the integrity and functions of cerebral endothelial cells induced by ZIKV could be related to loss of integrity in the BBB and further complications observed during infections.

REFERENCES

1. Cao-Lormeau, V., Roche, C., Teissier, A., Robin, E., Berry, A., Mallet, H., Sall, A. and Musso, D. (2014). Zika Virus, French Polynesia, South Pacific, 2013. Emerging Infectious Diseases. 20, 6.1084-1086.
2. Daneman, R. (2012). The blood-brain barrier in health and disease. Annals of Neurology. 72, 5. 648-672.
3. Luissint, A., Artus, C., Glacial, F., Ganeshamoorthy, K. and Couraud, P. (2012). Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. Fluids and Barriers of the CNS. 9, 1. 23.
4. Miner, J. and Diamond, M. (2016). Mechanisms of restriction of viral neuroinvasion at the blood–brain barrier. Current Opinion in Immunology. 38.18-23.
5. Oehler, E., Watrin, L., Larre, P., Leparc-Goffart, I., Lastere, S., Valour, F., Baudouin, L., Mallet, H., Musso, D. and Ghawche, F. (2014). Zika virus infection complicated by Guillain-Barre syndrome – case report, French Polynesia, December 2013. Euro Surveillance. 19, 9. 20720.

Mitochondrial and metabolic alterations in CD4 T cell during the acute phase of trypanosoma cruzi infection

Yamile Ana, Jorge David Rojas Marquez, Laura Fozzatti, Fabio Marcelo Cerbán, Cinthia Carolina Stempin

Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI CONICET), Cordoba, Argentina
yana@fcq.unc.edu.ar; drojas@fcq.unc.edu.ar; lfozzatti@fcq.unc.edu.ar; fcerban@fcq.unc.edu.ar; cstempin@gmail.com

Keywords: Infection, Metabolism, Trypanosoma cruzi, CD4 T cells, Mitochondrial ROS

Trypanosoma cruzi, the parasite that causes Chagas disease, was identified 100 years ago. However, this infection is still a serious social and public health problem being endemic in Central and South America. Traditionally, it has been limited to poor rural areas where vector transmission is the main route of infection. Migration from rural to urban areas, congenital transmission and transplants have allowed the disease to spread beyond its natural geographical boundaries, becoming a global health issue. The disease involves an acute stage that evolves to a chronic stage that is usually asymptomatic, however one third of patients develop dilated cardiomyopathy that can progress to heart failure due to the effects of parasite persistence, immune dysregulation and microvascular damage.

Several alterations of the immune response have been described during acute phase of the infection, including a dysfunction in the T cell compartment due to the generation of unresponsive anergic T cells and induction of CD4 T cell apoptosis causing deletion of effector cells favoring the persistence of the parasite. However, despite several decades of research, the mechanisms underlying the immunoregulation induced by the parasite are only partially understood and by operating simultaneously they make the response inefficient.

We recently demonstrated that CD4 T cells from acute phase of T. cruzi experimental infection showed an increase in the E3-Ubiquitin-Ligase Gene Related to Anergy in Lymphocytes (GRAIL) expression coinciding with a decreased proliferation in these cells. In addition, CD4 T cell displayed as well increased PD-1 expression, reduced IL-2 production and lower mTOR activation. It has been shown that upon activation T cells undergo metabolic reprogramming to glycolysis and mitochondrial biogenesis required to support their functions. Since mTOR is a central regulator of metabolism, we investigate the status of metabolic pathways in CD4 T cells during

T. cruzi infection. BALB/c mice were infected by intraperitoneal injection with 500 trypomastigotes of Tulahuen strain. CD4 T cells were purified from spleen of uninfected (control) or infected mice at different days post infection (d.p.i.). We evaluated expression of nutrient transporters CD98 (aminoacids) and Glut1 (glucose) as well as uptake of a fluorescent glucose analog 2NBDG by Flow cytometry. We did not observed differences in CD98 and Glut1 expression in ex vivo CD4 T cells from infected animals compared with controls. However, after anti-CD3/anti-CD28 stimulation, CD4 T cells from control and 42 d.p.i animals were able to upregulate these transporters besides increased the uptake of 2NBDG compared to unstimulated cells. However, CD4 T cells from 21 d.p.i. animals showed no differences compared to unstimulated cells. Additionally, to study mitochondrial function we combined a potential-dependent (MitoOrange) and a potencial-independent mitochondrial dye (MitoGreen) to identify CD4 T cells with depolarized mitochondria as well as measured mitochondrial ROS production (MitoSox) by Flow cytometry. At day 15 d.p.i. a higher fraction of CD4 T cells had depolarized mitochondria and produced increased levels of mROS compared with control or 42 d.p.i. animals, being mROS production greater in CD4 T cells with high PD-1 expression. These results may indicate T. cruzi induces mitochondrial alteration with high production of mitochondrial ROS leading to metabolic dysregulation of CD4 T cells. Then this mechanism could contribute to the immunosuppression observed during the acute phase of T. cruzi infection.

Efect of tamoxifen on the immune response of CBA/Ca mice infected with Plasmodium berghei ANKA

Luis Antonio Cervantes Candelas, Jorge Morales Montor, Martha Legorreta Herrera

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
cervantescandelasluis@gmail.com; jmontor66@biomedicas.unam.mx; marthal@unam.mx

Keywords: Cells, Malaria, Tamoxifen, Citokins, 17b-oestardiol

Introduction: Malaria is a infectious disease caused by the parasite Plasmodium. In 2016 it generated 216 million of new cases and 445 thousand of deaths. The incidence of both sexes is the same, but the intensity of symptoms and mortality is higher in men than in women. Given that sexual hormones and the interaction with their receptors are responsible for the main differences between sexes, it is possible that 17β-estradiol is involved in this sexual dimorphism. Dendritic cells and macrophages initiate the immune response via phagocitosis of parasitised erythrocytes these cells process and present parasite antigens that promote maturation of virgin T cells (CD3+) to CD4+ or CD8+ T cells. In addition, NK cells (CD16+/CD56+) and B lymphocytes (CD19+) also contribute to complete elimination of Plasmodium in the spleen. To stimulate lymphocyte maturation and cell activation are required cytokines such as INF-γ, TNF-α, IL-2, IL-4, IL-6, IL-17 e IL-10. Gonadectomy increases: parasitemia, the number of CD3+ T and CD19+ B lymphocytes in female mice infected with P. berghei ANKA. 17^2-estradiol administration activates regulatory T lymphocytes and macrophages and inhibits the proliferation of NK cells. However, is unknown if 17β-estradiol regulates the immune response in Plasmodium infections. Thus, in this work, we blocked estrogen receptors with tamoxifen in female CBA/Ca mice infected with P. berghei ANKA, we quantified parasitaemia, the amount of CD3+, CD4+, CD8+, CD19+, CD107b+ and CD16+/56+ cells in the spleen, and cytokines INF-γ TNF-α, IL-2, IL-4, IL-6, IL-17 e IL-10 in serum. In addition, we evaluated the concentration of progesterone, dehydroepiandrosterone (DHEA), testosterone and 17^2- estradiol in sera.

Methodology: Female CBA/Ca mice were treated with tamoxifen or vehicle, half of the mice in each group were infected with P. berghei ANKA, a group of mice without treatment was included. After day 4 post-infection, parasitaemia was evaluated diary by optical microscopy. All mice were sacrificed at day 8 post-infection, CD3+, CD4+, CD8+, CD19+, CD107b+ and CD15/56+ cells were assessed in spleen. In addition,

INF-γ, TNF-α, IL- 2, IL-4, IL-6, IL-17 e IL-10 in serum was quantified by flow cytometry, the concentration of progesterone, testosterone, DHEA and estradiol in the serum was quantified by EIA.

Results and Discussion: Treatment with tamoxifen significantly increased parasitaemia from day 5 post-infection in relation to the group of mice treated with vehicle or the group of mice without infection. In addition, the administration of tamoxifen decreased the levels of IL-2, IL-4, IL-6, INF-γ and CD8+, CD19+ and NK cells. This could be due to the fact that the lack of interaction of 17 ²-estradiol with its receptor decreased the concentration of cytokines IL-2, IL-4, IL-6, INF-γ, preventing cells from maturing and decreased the number of CD8+, CD19+ and NK cells in spleen corresponding to the increase in parasitaemia. The group of infected mice without treatment developed higher serum concentration of progesterone, testosterone and DHEA, while the concentration of 17²-estradiol decreased compared to the group of mice treated with tamoxifen or treated with vehicle.

Conclusion: The results show that 17β-estradiol regulates the immune response in malaria and helps explain at least in part the sexual dimorphism that occurs in the disease.

A role for IL-10 in the expansion and functional activation of CD8+ T cells during acute Trypanosoma cruzi infection

Agustina Maria Pino Martinez[1,2], Cristian Gabriel Miranda[1,2], Estela Ines Batalla[1,2], Stella Maris González Cappa[1,2], Catalina Dirney Alba Soto[1,2]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina
agus_mpm@hotmail.com; cristian_gam@gmail.com; estelab@yahoo.com.ar; smgcappa@gmail.com; catalina.alba@gmail.com

Keywords: Trypanosoma cruzi, CTL, t cell exhaustion, Interleukin 10 (IL-10), CD8 T cell expansion

Chagas disease is a potentially life-threatening illness caused by the intracellular protozoan parasite Trypanosoma cruzi (T. cruzi). Among T. cruzi chronically-infected individuals, 35% will develop mild- to severe cardiac and less frequently digestive forms of the disease and the rest of them will show no signs or symptoms of illness. It is currently estimated that 6–7 million people are infected with the addition of 56,000 new cases per year and 12,000 deaths. Yet, the migratory waves between Latin America and wealthy countries have spread the disease to North America, Europe, and the Western Pacific. According to WHO estimations for 2011, 300,000 T. cruzi infected people resided in the United States, 90,000 in Europe, 1,500 in Australia and 3,000 in Japan.

IL-10 is a pleiotropic cytokine with immunoregulatory functions that affect diverse cell populations. Previous work from our group has demonstrated the participation of IL-10 in immunoregulatory mechanisms launched at the interphase between innate and adaptive immunity against T. cruzi (Alba Soto CD et al. Vaccine. 2010; Poncini CV et al. Mol Immunol. 2010; Batalla EI et al. J Innate Immun. 2013; Poncini CV et al. Infect Immun. 2008). In a model of experimental infection with the protozoan T. cruzi we found augmented morbidity and lower parasite control in IL-10 deficient mice (IL-10 KO) compared to wild-type (WT) mice. To understand the increased susceptibility of T. cruzi infected IL-10 KO mice, we evaluated the performance of main effector mechanisms involved in parasite control. Despite of a Th1 balanced cytokine profile, enhanced macrophage function and dendritic cell activation IL-10 KO mice were more susceptible to infection. However, the kinetics of T cells in spleen and peripheral blood revealed that infected IL-10 KO mice failed to increase the number of spleen and circulating total CD8+ T cells, a phenomenon usually observed from the second week of infection in WT mice. Total CD8+ T cells from IL-10 KO mice

exhibited diminished proliferation, cytotoxic potential, IFN-γ production and lower survival than their WT counterparts. We also studied the impact of IL-10 on the T. cruzi-specific response of CD8+ T cells. For this, we measured in vivo the cytotoxicity of CD8+ T cells that recognize an MHC-I –restricted epitope from T. cruzi trans-sialidase, IYNVGQVSI. Lysis of target cells by parasite-specific CD8+ T cells was lower in IL-10 KO mice than in WT mice thus reflecting that the effect of IL-10 absence on CD8+ T cell function encompassed total as well as T. cruzi-specific CD8+ T cells. Moreover, the absence of IL-10 selectively affected expansion, survival and PD-1 expression of CD8+ T cells without affecting these same parameters on CD4+ T cells. CD8+ T cells from IL-10 KO infected mice exhibited a phenotype similar to that of exhausted CD8+ T cells. The participation of IL-10 in the primary expansion and functional activation of CD8+ T cells that characterizes acute in vivo infection was unexpected. In fact, the anti-inflammatory role of IL-10 has been extensively explored, while its stimulatory role has received less attention. Stimulatory properties of IL-10, including those that influence CD8+ T cells as the ones shown have been described. Accordingly, this cytokine increase their proliferation and cytotoxicity against non-specific and anti-gen-specific stimuli, viability during proliferation as well as the frequency of CD8+ T-cell precursors. Collectively, these findings reveal that during acute infection, IL-10 plays a previously unrecognized stimulatory role on CD8+ T cells, the most relevant lymphocyte population for the control of intracellular T. cruzi stages. Considering that the capacity to produce IL-10 can be genetically established, these results emphasize the significance of our findings for human infection. The profile of the CD8+ T cell response has a central role in the severity of human T. cruzi infection and the control of T. cruzi in sites of parasite persistence. A clear knowledge of the mechanisms that drive effector functions of this cytotoxic cell population is critical to understand factors involved in pathogen persistence and for the rational design of prophylactic strategies against T. cruzi.

Brucella abortus RNA is the vita-PAMP employed by this bacterium to inhibit the IFN-γ-induced Major Histocompatibility Complex (MHC) molecules surface expression, evading the host immune response

Maria Ayelen Milillo[1], Aldana Trotta[1], Luciana Balboa[1], Guillermo Hernán Giambartolomei[2], Paula Barrionuevo[1]

[1]Academia Nacional de Medicina, Buenos Aires, Argentina
[2]Instituto de Inmunología, Genética y Metabolismo (INIGEM-CONICET), Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, Buenos Aires, Argentina
ayelen_milillo@hotmail.com; aldanatrotta@hotmail.com; luciana_balboa@hotmail.com; ggiambart@ffyb.uba.ar; pbarrion2004@yahoo.com.ar

Keywords: Brucella abortus, RNA, Chronic infection, MHC Down-Modulation, Brucella Lipoproteins, Immune Evasion Strategies

Brucella abortus is an intracellular pathogen capable of surviving inside macrophages. In order to persist inside the host and establish a chronic infection, this bacterium must trigger different strategies to evade the robust adaptive T cell response it elicits. Previously, we demonstrated that B. abortus inhibits the IFN-γ-induced surface expression of MHC-II on human monocytes. As a consequence, the infected macrophages show a reduction in antigen presentation to TCD4+ lymphocytes. B. abortus outer membrane lipoproteins –a bacterial structural component- are involved in MHC-II down-modulation through the secretion of IL-6. Moreover, they are responsible for inhibiting the IFN-γ-induced transcription of the Class II Transactivator and MHC-II genes. Accordingly, we demonstrated that B. abortus-infected monocytes show a drastic reduction of MHC-II expression either in the surface or within the cell. Nevertheless, MHC-II down-modulation by B. abortus lipoproteins was less marked than the one observed with the infection. This last evidence led us think that there should have been another component associated to live bacteria implicated in MHC-II down-modulation. In fact, we have lately showed that B. abortus RNA, a PAMP associated to bacterial viability or vita-PAMP, is the component involved in MHC-I down-modulation. Inhibition of MHC-I expression was not due to changes in protein synthesis. Rather, this phenomenon results from the capacity of B. abortus RNA to retain the MHC-I molecules within the Golgi apparatus. In addition, we also discovered that the pathway associated with this event is the EGFR's as EGF-like ligands could also reproduce MHC-I down-modulation observed in monocytes infected with B. abortus or stimulated with its RNA. Thus, the aim of this study was to

analyse whether B. abortus RNA could also contribute to MHC-II down-modulation mediated by B. abortus. For this, human monocytic THP-1 cells were incubated with B. abortus RNA (1–10 µg/ml) in the presence of IFN-γ for 48 h. The expression of MHC-II molecules (HLA-DR) was then evaluated by flow cytometry. B. abortus RNA as well as RNase I-treated RNA significantly ($p < 0.001$) down-regulated the IFN-γ-induced surface expression of MHC-II molecules in a dose-dependent fashion. This last result implicates that not only does RNA down-modulate MHC-II expression but also RNase digestion products. This phenomenon was not due to loss of cell viability as we did not observe significant apoptosis in RNA-treated THP-1 cells. Furthermore, this event was also reproduced in peripheral blood human monocytes ($p < 0.05$). Accordingly, when THP-1 cells were stimulated with B. abortus lipoprotein (L-Omp19) and RNA, MHC-II down-modulation was even higher than that observed with merely RNA or L-Omp19 ($p < 0.01$). Also, eukaryotic RNA was not capable of inhibiting IFN-γ-induced MHC-II expression. Interestingly, the expression of co-stimulatory molecules CD80, CD86 and CD40, which are also up-regulated by IFN-γ, were significantly stimulated on RNA-treated THP-1 cells ($p < 0.05$; $p < 0.001$ and $p < 0.001$, respectively). This last rest result shows that the inhibition of MHC molecules is not a global effect on other IFN-γ-induced molecules. With regard to the signalling pathway, we could also demonstrated that stimulation of THP-1 cells with EGF was able to reproduce MHC-II down-modulation mediated by B. abortus RNA ($p < 0.01$), indicating that EGFR pathway is involved in this phenomenon. By confocal microscopy, we also demonstrated that B. abortus RNA mimics what was observed with the infection: a diminished MHC-II expression on RNA-treated THP-1 cells. Actually, on the cells in which we observed MHC-II, it does not colocalize with Golgi apparatus. In conclusion, B. abortus employs different strategies to conceal itself inside macrophages and evade host immunity. Specifically, its RNA is a vita-PAMP used by the bacteria to inhibit MHC molecules expression. B. abortus RNA, together with its lipoproteins, succeed in making the bacteria establish a chronic infection.

Is adrenal cortex from TB patients a source of cytokines which contributes to get independence from HPA axis?

Estela Isabel Bini[1], María Irene Cerezo Cortés[2], Brenda Marquina Castillo[1], Ricardo Marquez Velasco[3], Natalia Santucci[4], Armando Gamboa Domínguez[1], Maria Luisa Bay[4], Oscar Adelmo Bottasso[4], Rogelio Hernandez Pando[1]

[1]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[2]National University of Colombia at Bogotá, Bogotá, Colombia
[3]Instituto Nacional de Cardiología, Mexico City, Mexico
[4]National University of Rosario, Rosario, Argentina
estelabini@yahoo.com.ar; micerezoc@unal.edu.co; brenda.marquina@gmail.com; marquezric@hotmail.com; nesantucci@gmail.com; agamboad@gmail.com; bay.mahuad@yahoo.com.ar; oscarbottasso@yahoo.com.ar; rhdezpando@hotmail.com

Keywords: Adrenal Glands, Cytokines, HPA axis, tuberculosis (TB), glucocoricoids

Introduction: The chronic nature of tuberculosis (TB) and the protracted immuno-inflammatory reactions are implied in a series of metabolic and immune-endocrine changes accompanying the disease.

In humans, TB induces chronic stress and the activation of neuro-hormonal circuits such as the hypothalamous-pituitary-adrenal (HPA) axis or the adrenergic nervous system, which leads to increased glucocorticoids (GC) and cathecolamines levels. Nevertheless, increased GC levels cannot only be explained by the HPA axis activation since that corticotropin-releasing hormone and adrenocorticotropin hormone levels remains low. This could suggest an independent pathway from de HPA axis for GC production.

The activation of TLRs expressed in adrenal cortex cells can induce cytokines production in sepsis. This may be the pathway that regulates the synthesis of adrenal steroids in TB patients. In a previous work carried out in testis from TB patient necropsies, we explored how components from the hypothalamous-pituitary-gonadal axis and cytokines are involved in disease immunopathology. Here, we analyzed the adrenal cortex from these necropsies in the aim to determine the production of cortisol, DHEA and diverse cytokines by adrenal TB patient glands.

Methods and Materials: Adrenals from 14necropsies of patients dying from TB and fivepatients dying from cardiovascular diseases (no TB) as controls were studied by immunohistochemistry to detect Cortisol, DHEA, IL1β, IL1β- Receptor, IL6, TNFα, TGFβ, IFNγ and IL10. PCR analysis was performed in order to determine the presence of correspondent mRNAs.

Results: Adrenals necropsies from TB patients exhibited positive staining to cortisol and the studied cytokines, exhibiting IL6 and TNFα particularly strong immunostaining in the cortical cells from the reticular and fascicular zones. The expression of DHEA decreased in TB patients, in comparison, adrenals from control patients exhibited increased DHEA levels. Adrenals from controls revealed slight or negative immunostaining for cortisol and cytokines. These results were confirmed by PCR analysis.

Conclusions: Cortisol and pro-inflammatory cytokines, are produced by cells from adrenal TB patient glands. That could regulate the production of steroid hormones by passing the central control.The persistence of the inflammatory process observed in TB patients may be responsible of the generation of new regulatory mechanisms, independent from the HPA axis. Despite central mechanisms of HPA still remain functional; in the case of a severe infectious disease as TB, an intra-adrenal mechanism of control may play an important rol in the immune-endocrine regulation.

MLIF administration improves cerebral malaria in Plasmodium berghei ANKA infected C57BL/6 mice

Luvia Enid Sánchez-Torres[1], Adrian Galán-Salinas[1], Jackeline Pérez-Vega, Raúl Silva[2], Luis Fabila-Castillo[1]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico

luviasanchez@hotmail.com; agalan88@hotmail.com; grove_jacke@hotmail.com; silgarrul@yahoo.com.mx; lfabila@ipn.mx

Keywords: Cytokines, mouse model, Blood Brain Barrier (BBB), cerebral malaria, MLIF

In 2016, an estimated 216 million cases and 445 000 deaths occurred worldwide due to malaria. In humans, P. falciparum represents 99% of the cases besides being the species that causes the most severe form of malaria. Cerebral malaria (CM) is a devastating complication which manifests with an increase in pro-inflammatory cytokines, endothelial cell activation with adhesion of leucocytes, platelets, monocytes and infected red blood cells (iRBC) leading to microcirculation obstruction, hypoxia, and blood-brain barrier disruption, causing haemorrhage, coma and in most cases death. Although not completely identical to the human disease, animal models have complimented the understanding pathogenesis of CM. The most established model is the infection of susceptible mice C57BL/6 with Plasmodium berghei ANKA (PbA). In this model of Experimental Cerebral Malaria (ECM), at least 60% of susceptible mice develop neurological symptoms culminating with the death of the 100% of the animals around 7–9 days after infection. Considering the exacerbated inflammatory response that occurs in CM, the effect of the Monocyte Locomotion Inhibitor Factor (MLIF), a pentapeptide with anti-inflammatory properties was evaluated. We used a 6-dose administration scheme from day 4 to day 7 after infection; MLIF increased survival without neurological signs of ECM, the integrity of the blood-brain barrier was not compromised showing less iRBC sequestration in brain microvasculature and a downregulation of IFN-γ, TNF and IL-10 production in serum. However, mice die from anaemia with high parasitemia after day 20 post-infection. In conclusion, MLIF increases PbA-infected C57BL/6 mice survival, preserves the integrity of the BBB and prevents the increase of cytokines disease-related.

The role of HS1 in vascular permeability and PMN recruitment in the lung during sepsis

Idaira Maria Guerrero Fonseca, Alexander García Ponce, Eduardo Vadillo Rosado, Porfirio Nava, Michael Schnoor

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
idairaguerrerof@gmail.com; Alexgp0906@gmail.com; evadillo@hotmail.com; pnava@cinvestav.mx; mschnoor@cinvestav.mx

Keywords: Neutrophils, Sepsis, intravital microscopy, cecal ligation and puncture, Endothelial permeability, Hs1

Background and Aim: Sepsis is defined as a life-threatening multiorgan dysfunction caused by dysregulation of the host immune response to infection. This syndrome represents one of the most important health problems in intensive care units worldwide; and in Mexico reaches a mortality rate of 30%. This is due to failure in the development of efficient therapies to improve all pathological conditions. Multiorgan failure and acute respiratory distress syndrome (ARDS) are the most important complications. The excessive activation and recruitment of polymorphonuclear leukocytes (PMN) contribute to tissue damage and organ failure during severe sepsis. Both processes are triggered by the constant reorganization of the actin cytoskeleton which is regulated by actin-binding proteins (ABP) such as the hematopoietic lineage cell-specific lyn substrate 1 protein (HS1). It has recently been shown that HS1 is important for trans-endothelial migration (TEM) of PMN because absence of this protein results in reduced activation of GTPases and integrins and consequently less chemotaxis and transmigration during inflammation. Therefore, we evaluated the role of HS1 in PMN recruitment and lung damage during an in vivo model of murine sepsis.

Methods: Male C57BL/6 WT and C57BL/6 HS1 KO mice were used to induce sepsis by cecal-ligation and puncture (CLP). Plasma levels of the cytokines TNF-α and IL-1β, IL-6 and IL-10 were measured with Milliplex Magpix to assess systemic inflammation. To determinate the severity of lung damage, histology was analyzed by hematoxylin/eosin stainings and apoptosis was analyzed by western blot of cleaved PARP protein levels. The interactions between endothelial cells and leukocytes during sepsis-induced TEM were analyzed using intravital microscopy (IVM) in the cremaster muscle. The rate of leukocyte recruitment into the lung was assessed using flow cytometry. Endothelial permeability in the lung was investigated via leakage of 2μm fluosphere beads after tail vein injection by fluorescence microscopy. Additionally, 3kDa cascade blue-dextran,

10kDa fluorescein-dextran and 40kDa Texas Red-dextran were administered by tail vein injection to evaluate the size of the gaps in the vascular endothelium.

Results and Conclusions: Septic mice were monitored for five days after CLP to analyze their health status and behavior. HS1 KO mice showed significantly improved survival compared to WT mice suggesting that HS1 KO mice are protected from CLP-induced sepsis. Accordingly, histological analyses of lung tissues showed a little damage and inflammatory response in HS1 KO animals 24 hours after CLP. Moreover, apoptosis in the lungs of septic HS1 KO mice was reduced as indicated by lower levels of cleaved PARP. No significant differences were found in the plasma levels of the proinflammatory cytokines TNF-α, IL-1β and IL-6 between WT and HS1 KO. By contrast, the concentration of IL-10 was higher in septic HS1 KO mice. This anti-inflammatory cytokine is critical for the control of the immune response during sepsis, and it could be supporting the survival of HS1 KO mice. As expected, IVM and flow cytometry of lung tissues revealed that less PMN were recruited into the lungs and cremaster muscles in HS1 KO mice. This reduction in excessive PMN recruitment may contribute to the observed reduced tissue damage in HS1 KO mice. However, no differences were found in endothelial permeability in the lungs of HS1 KO mice compared with WT. Our study demonstrates that HS1 deficiency diminished sepsis-induced lung damage and mortality through increased IL-10 production and reduced apoptosis and PMN recruitment.

Dual RNA sequencing of human splenic macrophages infected with Mycobacterium tuberculosis clinical isolates reveals distinct macrophage and bacterial transcriptomes

Victor A Lopez-Agudelo[1], Andres Baena[1], Vianey Barrera[2], Juan P Isaza[1], Felipe Cabarcas[1], Rigoberto Rios[1], Juan F Alzate[1], Luis F Barrera

[1]Universidad de Antioquía, Medellín, Colombia
[2]National University of Colombia at Medellin, Medellin, Colombia
viclopezag@gmail.com; andres.baenag@udea.edu.co; vpbarrerae@unal.edu.co; juanpabloisaza@gmail.com; fcabarcas@gmail.com; rigoberto.rios@udea.edu.co; jfernando.alzate@udea.edu.co; luis.barrera@udea.edu.co

Keywords: Transcription, Genetic, innate immune response, RNA-seq analysis, Human macrophage, M. tuberculosis infection

Introduction: Tuberculosis (TB) disease is the leading cause of death worldwide by a bacterial infection1. TB is caused by Mycobacterium tuberculosis (Mtb), a bacillus that resides and replicates in tissue macrophages, most frequently in alveolar macrophages (AMs)2. However, under some circumstances, Mtb disseminates to other organs in the body leading to extra-pulmonary TB3. The transcriptomic consequences of Mtb infection of human AMs are almost unknown, and no data are presently known on the consequences of Mtb infection in extra-pulmonary human macrophages. We have been using human splenic macrophages (hSMs)4,5 to explore the early gene expression profile of Mtb clinical isolates of the LAM (Latin American Mediterranean) family obtained from TB patients from the city of Medellín, Colombia, as a model to explore the transcriptomic response of both the infected macrophage and the infecting Mtb bacillus.

Methods: hSMs obtained from spleen mononuclear cells from deceased patients4 (Ethics Committee approval and written informed consent) were infected (MOI 10:1) for 6 hours with the clínical isolates of Mtb, UT127, and UT205, belonging to the LAM09 family of Mtb. This family of Mtb is associated with the frequently active form of pulmonary TB in the city of Medellín, Colombia6. RNA-seq libraries were prepared from high-quality RNA (RIN>8) and sequenced using an Illumina protocol (Macrogen, Korea). Analysis of transcriptomic data from infected SMs and Mtb isolates was performed using the EdgeR Bioconductor package7. For infected hSMs, gene enrichment was done using the full list of expressed genes and examined with GO-seq8.

Further analysis (Biological Process and KEGG pathways) on the immune response genes (ImmunPRO database) to UT205 and UT127 clinical isolates infection was done using the STRING and the GO Ontology databases. Over and under-represented gene categories were examined with PANTHER Overrepresentation Test (Released 20171205) (FDR<0.05). Mycobacterial transcriptomes were first upper (75) quartile normalized. Highly expressed genes were selected on the normalized count values greater than percentile 90. STRING and UniProtKB databases were further used to identify genes with closed related function and GO enrichment terms. Sets of unique and shared genes between UT127 and UT205 were obtained to find differences used for each strain in response to the macrophage environment.

Results: The genomes of UT127 and UT205 are more than 99% identical. However, the transcriptomic response of the hSMs to these Mtb strains display important differences. From a total of 1600 immune response associated genes (ImmunPRO), 462 (40.7%) are shared by both isolates while UT127 uniquely express 539 (47.5%) and UT205 uniquely express 134 (11.8%). Thus, infection of hSMs with UT127 induces a more potent innate response as compared to UT205. Indeed, the most relevant biological processes (GO) including immune response process as well as KEGG pathways display a more significant false discovery rate (FDR<0.05) value for all functional categories and pathways in UT127 infection compared to UT205. For example, 16/1952 (0.82%) and 11/1952 (0.56%) of biological processes involving type 1 IFN and type 2 IFN, respectively, are significantly enriched in response to UT127 infection while 3/224 (1.33%) of biological processes involves type 1 IFN in response to UT205 and no biological process involving type 2 IFN is enriched. Interestingly, several biological processes involving cell death (apoptosis or necrosis), are significantly enriched in response to UT127 infection, and no one of such processes are significantly enriched in response to UT205 infection. Initial analysis indicates that both clinical isolates displayed a distinct transcriptome profile in response to hSMs infection. GO terms such as "response to metal ion", "response to stimulus" and "inorganic substances" were enriched for the intracellular UT127, including genes associated to metal ion acquisition (e.g., mbtB, mbtD, mbtC, mbtE, mbtF), and detoxification (ctpC, ctpV and csoR) while intracellular UT205 preferentially expressed genes associated with categories such as "growth" (rplQ, rplF, rplW, rpsS, rpsC, rplV, rplM, rplO, rpsE, rplK), "primary metabolism", "lipid metabolism" (kasB, papA1, papA2, fadA6, fadD2, echA20) and "cholesterol metabolism" (kshA, fadD3).

Conclusions: Human tissue macrophages express a Mtb strain-specific transcriptome that may be a consequence of the particular Mtb strain-specific adaptation to the intracellular environment posed by the macrophage. While the stronger innate immune

genes response to one clinical isolate may suggest an early and more potent recognition by the innate immune system, a less intense recognition by the macrophage may lead to a delayed innate immune response. Thus, two "virulence strategies" may be used by Mtb: a stress-related one that may lead to a faster proliferation of the bacteria, and a second one, "hiding" strategy, that may evade an efficient, immediate recognition by the innate immune system and let the bacteria to stay unrecognized by a longer period. The severity of TB disease may vary accordingly to those two strategies.

The tuberculous pleural effusion alters the metabolic reprogramming of M1 activated macrophages

José Luis Marín[1,2], Melanie Genoula[1,2], Gabriel Duette[3], Malena Ferreyra[1,2], Geanncarlo Lugo-Villarino[1,4], Olivier Neyrolles[1,4], Eduardo José Moraña[5], Domingo Palmero[5], Maria Del Carmen Sasiain[1,2], Pablo Schierloh[1,2], Luciana Balboa[1,2]

[1]Laboratoire International Associé (LIA), Toulouse, France
[2]Instituto de Medicina Experimental, Academia Nacional de Medicina (CONICET), Ciudad autónoma de Buenos Aires, Argentina
[3]Institute of Biomedical Investigations in Retrovirus and Aids (INBIRS), Buenos Aires, Argentina
[4]UMR5089 Institut de Pharmacologie et de Biologie Structurale (IPBS), Toulouse, France
[5]Instituto de Tisioneumonología Raúl F. Vaccarezza, Buenos Aires, Argentina
Joseluismarinfranco@gmail.com; mgenoula@gmail.com; gabriel.duette@gmail.com; malenaferreyra@gmail.com; geanncarlo.lugo@ipbs.fr; olivier.neyrolles@ipbs.fr; ejm2878@hotmail.com; djpalmero@intramed.net; mariadelcarmen.sasiain@hotmail.com; lp_shier@hotmail.com; luciana_balboa@hotmail.com

Keywords: aerobic glycolysis, M1 macrophages, metabolic reprogramming, Tuberculous pleural effusion, HIF-1α expression

Classically activated or M1 macrophages are key players of the first line of defense against bacterial infections such as Mycobacterium tuberculosis (Mtb), the etiological agent for tuberculosis (TB). Mtb infection remains a major health problem worldwide, with up to 2 billion people chronically infected. The chronic host-pathogen interaction in TB leads to extensive metabolic remodeling in both the host and the pathogen1. In fact, the success of Mtb as a pathogen derives from its efficient adaptation to the intracellular milieu of human macrophages, displaying several strategies to circumvent the microbicidal activity in these cells. Normally, in response to infection, activation of the host immune cells is accompanied by a switch in the bioenergetic pathway from oxidative phosphorylation to aerobic glycolysis, which is required for the production of antimicrobial and pro-inflammatory effector molecules2,3. In this regard, M1 macrophages are known to obtain energy through glycolysis, displaying an increase in glucose uptake as well as the conversion of pyruvate to lactate4. A key transcription factor orchestrating the expression of glycolytic enzymes is the hypoxia-inducible factor-1 (HIF1), a heterodimer comprised of α (HIF-1α) and β (HIF-1β) subunits, being HIF-1α the regulated component of the complex5. The activation of HIF-1α results in the production of pro-inflammatory cytokines and other mediators of the M1 phenotype, such as glycolytic enzymes and the glucose transporter GLUT16. Moreover, at least two interruptions in the Krebs cycle were described in M1

macrophages, leading to the accumulation of intermediates such as succinate. Succinate was known to regulate the stability of HIF-1α driving a sustained production of the pro-inflammatory cytokine IL-1β7,8. Since M1 macrophage effector functions are deeply related to the metabolic program in course, we hypothesize that Mtb can perturb the metabolic reprogramming in M1 in order to facilitate its intracellular persistence. In this regard, previous reports have demonstrated that the infection with Mtb leads to glycolysis in bone-marrow derived macrophages9,10, in lungs of infected mice11, and in lung granulomas from patients with active TB12. Of note, it has shown that Mtb infection induced the increase of HIF-1α expression in IFN-γ-activated macrophages which is essential for IFN-γ-dependent control of infection13. Based on this knowledge, we decided to study whether the local microenvironment of the infection modulates the metabolic reprogramming in M1 macrophages and whether it impact on host defense against Mtb. To assess this issue, we polarized human M1 macrophages with IFN-γ and LPS, and then treated them with the acellular fraction of tuberculous pleural effusion (TB-PE), which is genuine TB-derived microenvironment containing multiple soluble factors released locally during the infection. We found that the treatment with TB-PE increased the expression of the glucose transporter GLUT1, as well as the uptake and consumption of glucose in M1 macrophages. Surprisingly, the release of lactate was reduced in TB-PE-treated M1 macrophages, together with the expression of HIF-1α, the production of mitochondrial ROS, and the production of IL-1β. Finally, we demonstrated that the TB-PE-treated M1 macrophages contained higher bacillary loads. In conclusion, while TB-PE treatment promotes glucose uptake, this additional glucose is not used to fuel the aerobic glycolytic pathway associated with the microbicidal activity in M1 macrophages. Instead, aerobic glycolysis seems to be reduced by TB-PE in association with a reduced ability to control the bacterial growth. Therefore, we propose that soluble factors released locally during the infection alter the metabolic reprogramming of M1 macrophages impacting on their ability to control the infection. The soluble mediators responsible for this metabolic alteration are still under study. We consider that a better understanding of the molecular mechanisms underlying host-pathogen interactions could provide a rational basis for the development of effective anti-TB therapeutics.

Susceptibility to Toxoplasma gondii infection is modified by a previous Taenia crassiceps infection

Danae Mendoza-Sánchez, Imelda Juárez-Avelar, Alexia Sánchez-Arteaga, Tonathiu Rodriguez, Miriam Rodriguez-Sosa

Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico

mendoza.sa.danae@gmail.com; imelda_juarez@yahoo.com; dalexia.02@hotmail.com; tonathiurh@hotmail.com; rodriguezm@unam.mx

Keywords: Helminths, Survival Rate, Toxoplasmosis, Co-infection, immune response polarization

Background: Great parasitic infections incidences exist in developing countries, among them, helminths and intracellular parasites are important etiologic agents. Helminth and Toxoplasma gondii (T. gondii) co-infections are frequent; nevertheless, the coexistence of these two parasites in the immune system is poorly understood.

Aim: Understand the effect that helminth T. crassiceps have in the course of a secondary infection by T. gondii.

Experimental Design: Female mice BALB/c were infected with 15 cysticerci of the helminth Taenia crassiceps (T. crassiceps) ORF strain by intraperitoneal (i.p.) injection. The first infection was made whit T. crassiceps, this parasite modify the cellular immune response. During the first 1-3 weeks, the infection developed a pro-inflammatory profile Th1 (high levels of IL-2, TNF-a and IFN-g), in the weeks 4-5 developed a mixed profile (IFN-g, IL-4 and IL-10) and subsequently, in the 6-10 weeks changed to an anti-inflammatory profile Th2 (IL-4 and IL-10). This allowed us to investigate if the modifications induced in a second infection, are related to specific moments in the previously established infection. The second infection with 40 cysts of T. gondii semi-virulent strain ME49, was performed i.p. in groups of 5 mice, in the week 1, 4, or 8 after infection with T. crassiceps. Controls, were healthy mice, or were only infected by T. crassiceps. Mice survival was monitored, and after death we quantified the cysts number in the brain, determined the presence of splenomegaly, performed spleen H&E histology to assess tissue integrity, and quantified the amount of cytokines IFN-y, TNF-a, IL-4 and IL-10 weekly in serum.

Results: Mice co-infected on the week 1 died early, while the co-infected mice in the week 4 or 8 have increased survival time, all in comparison with mice infected only by T. gondii. Spleens of the group co-infected in the weeks 4 and 8, show increased splenomegaly, and histologic studies revealed a loss in the white and red pulp compartmentation. The reduction in survival observed in the group co-infected at week 1, correlated with high levels of IFN-y and TNF-a, and decreased levels of IL-4 and IL-10, compared with the group infected by T.gondii only.

Conclusion: The susceptibility to T. gondii was modified by prior T. crassiceps infection. The susceptibility varied as a function of the time after the first infection, the co-infection at week 1 exacerbated the mice inflammatory-Th1 immune response, decreasing their survival time. On the other hand, in the weeks 4 and 8 co-infection groups, we observed a decrease the inflammatory Th1 immune response. These results suggest that the susceptibility to an infection is modified by the previous presence of others infectious agents, even when the pathogens are no related. The epidemiological implications of these findings must be studied further, in order to determinate their possible effect in public health.

Formation of Foamy macrophages by Tuberculous Pleural effusions is triggered by the IL-10/STAT3 axis through ACAT up-regulation

Melanie Genoula[1,2], José Luis Marín Franco[1,2], Maeva Dupont[2,3], Denise Kviatcovsky[1,2], Maria Ayelen Milillo[1], Pablo Schierloh[1,2], Eduardo José Moraña[4], Susana Poggi[4], Domingo Palmero[4], Dulce Adriana Mata-Espinosa[5], Erika González-Domínguez[6], Juan Carlos León Contreras[5], Paula Barrionuevo[1], Bárbara Rearte[1], Marlina Olyissa Córdoba Moreno[1], Adriana Fontanals[7], Agostina Crotta Asis[8], Gabriela Gago[8], Céline Cougoule[2,3], Olivier Neyrolles[2,3], Isabelle Maridonneau-Parini[2,3], Carmen Sánchez-Torres[6], Rogelio Hernández-Pando[5], Christel Vérollet[2,3], Geanncarlo Lugo-Villarino[2,3], María del Carmen Sasiain[1,2], Luciana Balboa[1,2]

[1]Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina

[2]Laboratoire International Associé (LIA), Toulouse, France

[3]UMR5089 Institut de Pharmacologie et de Biologie Structurale (IPBS), Toulouse, France

[4]Instituto de Tisioneumonología Raúl F. Vaccarezza, Buenos Aires, Argentina

[5]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

[6]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico

[7]Leloir Institute Foundation (FIL), Buenos Aires, Argentina

[8]CONICET Instituto de Biología Molecular y Celular de Rosario (IBR), Rosario, Argentina

mgenoula@gmail.com; joseluismarinfranco@gmail.com; maeva.dupont@ipbs.fr; denise.kv@hotmail.com; mililloayelen@gmail.com; lp_shier@hotmail.com; ejm2878@hotmail.com; sepoggi@intramed.net; djpalmero@intramed.net; dulmat@yahoo.com.mx; erikagd1@hotmail.com; jcleoncdf@gmail.com; pbarrion2004@yahoo.com.ar; barbararearte@yahoo.com.ar; molyco89@gmail.com; afontanals@leloir.org.ar; agostina.crotta.asis@gmail.com; gago@ibr-conicet.gov.ar; celine.cougoule@ipbs.fr; olivier.neyrolles@ipbs.fr; isabelle.maridonneau-parini@ipbs.fr; csanchez@cinvestav.mx; rhdezpando@hotmail.com; christel.verollet@ipbs.fr; geanncarlo.lugo@ipbs.fr; msasiain@hematologia.anm.edu.ar; luciana_balboa@hotmail.com

Keywords: Lipids, Tuberculosis, IL-10, ACAT, stat3, Foamy macrophages

Tuberculosis (TB) is a highly contagious disease caused by Mycobacterium tuberculosis (Mtb) infection. The ability of Mtb to persist in its human host relies on numerous immune evasion strategies, such as the deregulation of the lipid metabolism leading to the formation of foamy macrophages (FM). The formation of FM is caused by infectious agents through deregulation in the balance between the influx and efflux of lipids. The free cholesterol released from the endocytosed lipoproteins moves from lysosomes

to the endoplasmic reticulum, where it is either re-esterified to cholesteryl ester by the enzyme acylCoA:cholesterol acyltransferase (ACAT), which can then be stored in cytosolic droplets (lipid bodies) (1). Lipid body accumulation within leukocytes is a common feature in both clinical and experimental infections, especially in mycobacterial infections (2, 3). Mtb infection leads to the induction of FM, process which is promoted by several mycobacterial lipids (4, 5, 6, 7). This event enables the fusion between Mtb-containing phagosomes and lipid bodies resulting in an abundant supply of lipids for the pathogen (4) which enables Mtb to switch into a dormancy phenotype and to become tolerant to several front-line antibiotics (8). Besides, lipid bodies represent a secure niche in order to avoid bactericidal mechanisms such as respiratory burst (9). Concerning the impact of FM on the host immunity against Mtb, it was shown that human macrophages exposed to lipids prior to Mtb infection failed to produce TNF-α and to clear the infection (10, 11). Taking into account that FM generated prior to Mtb infection impair the host immune response, there is a keen interest to identify the host-derived cytokines released at the site of Mtb infection, and to understand how these signals contribute to FM differentiation and alter host defense against Mtb. In this regard, it is well known that different activation programs in macrophages driven by pro or anti-inflammatory cytokines are associated to changes in the lipid metabolism (12). Therefore, it is likely that host cytokines produced in response to Mtb infection contribute to lipids turnover promoting FM formation, and consequently lead to Mtb persistence. To model those soluble host-immune factors we employed a physiological relevant sample derived from active TB patients such as the acellular fraction of tuberculous pleural effusions (TB-PE). Then, we treated human M-CSF-driven macrophages with TB-PE in order to mimic a genuine microenvironment derived during Mtb infection. According to the pattern of staining of neutral lipids with Oil Red O (ORO), we observed that TB-PE treatment induced lipid bodies accumulation in macrophages to the same extent as Mtb infection or exposure to Mtb-derived lipids and that it was specific for TB-PE treatment in comparison to pleural effusions from patients with heart failure (HF-PE). This foamy phenotype induced by TB-PE was accompanied by higher abundance of cholesteryl esters and triacylglycerols and higher CD36 expression in comparison to HF-PE-treated macrophages. Besides, we showed that the foamy formation was dependent on ACAT activity, as judged both by the increase of ACAT expression after TB-PE treatment and by the prevention of lipid bodies accumulation in the presence of Sandoz, a specific inhibitor of ACAT. Noticeably, IL-10 depletion from TB-PE prevented the augmentation of all these parameters but not that of IL-1β, IL-6, IFN-γ, IL-4, or TNF-α. In line with it, the complementation of IL-10-depleted TB-PE with exogenous IL-10 restored the foamy phenotype in a dose-dependent manner. Additionally, macrophages treated with IL-10-depleted TB-PE showed smaller sized lipidic vacuoles than those exposed to non-depleted TB-PE when cells were observed by electron microscopy. Moreover, we found a positive correlation between the levels of IL-10 and the number of lipid-laden CD14+ cells among the pleural cells in TB

patients, demonstrating that FM differentiation occurs within the pleural environment. Additionally, we observed that the acquisition of the foamy phenotype was associated with immunosuppressive properties such as a higher expression of anti-inflammatory markers, like CD163, mannose receptor (MRC1), and PD-L1, and a lower expression of HLA-DR, high IL-10 release, low TNF-α production, impaired Th1 activation, and high bacillary loads. To our concern, we provide evidence for the first time that FM display a reduced ability to activate a recall Th1 response of specific anti-mycobacterial T cell clones. Downstream of IL-10 signaling, we found that the transcription factor STAT3 was activated by TB-PE, detecting its phosphorylated form by western blot and immunofluorescence microscopy, and its chemical inhibition with Stattic prevented the accumulation of lipid bodies and ACAT expression in macrophages. Finally, we confirmed our results comparing whether BMDM derived from WT or IL-10-KO mice differed in their propensity to accumulate lipid bodies in response to Mtb-derived lipids. IL-10 deficiency partially prevented foamy phenotype induced by Mtb lipids, which in turn could be reverted by the addition of exogenous IL-10.

Based on our findings, we propose a model for the modulation of FM in the context of a physiologically relevant microenvironment promoted by Mtb infection in which the axis IL-10/STAT3 promotes the accumulation of lipid bodies throughout ACAT up-regulation that is accompanied by an increase of CD36 and the acquisition of immunosuppressive properties such as a reduced induction of anti-mycobacterial Th1 clones, an enhanced production of IL-10 and a more permissive phenotype for bacillary growth.

In conclusion, our results provide additional mechanisms by which the environment created by the infection process can drive the foamy differentiation even in the absence of the pathogen such as we observed that uninfected macrophages can be driven into FM in the presence of IL-10 and a source of lipids. These uninfected lipid-rich cells can abrogate the host innate and adaptive cellular defense mechanisms, and when these cells become infected, they may further favor pathogen persistence. Therefore, this knowledge may contribute to the identification of host molecular pathways that could be modulated to the benefit of the patient. In this regard, a better understanding of the molecular mechanisms underlying host-pathogen interactions could provide a rational basis for the development of effective anti-TB therapeutics.

REFERENCES

1. Pol A, Gross SP, Parton RG. Review: biogenesis of the multifunctional lipid droplet: lipids, proteins, and sites. J Cell Biol (2014) 204(5):635-46. doi: 10.1083/jcb.201311051.
2. Cardona PJ, Llatjos R, Gordillo S, Diaz J, Ojanguren I, Ariza A, et al. Evolution of granulomas in lungs of mice infected aerogenically with Mycobacterium tuberculosis. Scand J Immunol (2000) 52(2):156-63.

3. D'Avila H, Melo RC, Parreira GG, Werneck-Barroso E, Castro-Faria-Neto HC, Bozza PT. Mycobacterium bovis bacillus Calmette-Guerin induces TLR2-mediated formation of lipid bodies: intracellular domains for eicosanoid synthesis in vivo. Journal of immunology (2006) 176(5):3087-97.
4. Peyron P, Vaubourgeix J, Poquet Y, Levillain F, Botanch C, Bardou F, et al. Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for M. tuberculosis persistence. PLoS pathogens (2008) 4(11):e1000204. doi: 10.1371/journal.ppat.1000204.
5. Russell DG, Cardona PJ, Kim MJ, Allain S, Altare F. Foamy macrophages and the progression of the human tuberculosis granuloma. Nat Immunol (2009) 10(9):943-8. doi: 10.1038/ni.1781.
6. Kim MJ, Wainwright HC, Locketz M, Bekker LG, Walther GB, Dittrich C, et al. Caseation of human tuberculosis granulomas correlates with elevated host lipid metabolism. EMBO Mol Med (2010) 2(7):258-74. doi: 10.1002/emmm.201000079.
7. Podinovskaia M, Lee W, Caldwell S, Russell DG. Infection of macrophages with Mycobacterium tuberculosis induces global modifications to phagosomal function. Cell Microbiol (2013) 15(6):843-59. doi: 10.1111/cmi.12092.
8. Daniel J, Maamar H, Deb C, Sirakova TD, Kolattukudy PE. Mycobacterium tuberculosis uses host triacylglycerol to accumulate lipid droplets and acquires a dormancy-like phenotype in lipid-loaded macrophages. PLoS pathogens (2011) 7(6):e1002093. doi: 10.1371/journal.ppat.1002093.
9. Singh V, Jamwal S, Jain R, Verma P, Gokhale R, Rao KV. Mycobacterium tuberculosis-driven targeted recalibration of macrophage lipid homeostasis promotes the foamy phenotype. Cell host & microbe (2012) 12(5):669-81. doi: 10.1016/j.chom.2012.09.012.
10. Dodd CE, Pyle CJ, Glowinski R, Rajaram MV, Schlesinger LS. CD36-Mediated Uptake of Surfactant Lipids by Human Macrophages Promotes Intracellular Growth of Mycobacterium tuberculosis. Journal of immunology (2016) 197(12):4727-35. doi: 10.4049/jimmunol.1600856.
11. Asalla S, Mohareer K, Banerjee S. Small Molecule Mediated Restoration of Mitochondrial Function Augments Anti-Mycobacterial Activity of Human Macrophages Subjected to Cholesterol Induced Asymptomatic Dyslipidemia. Front Cell Infect Microbiol (2017) 7:439. doi: 10.3389/fcimb.2017.00439.
12. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. Journal of immunology (2006) 177(10):7303-11.

Expression of CX3RCR1 in monocytes and effector memory CD8+ T cells in peripheral blood of women with cervical intraepithelial neoplastic-I

María Luisa Pita-López[1], Pablo Cesar Ortiz Lazareno[2], José Alberto Alcazar-Ríos[1], German Muñoz-Sanchez[1], Edder Adonay Corona-Godoy[1], Oscar Martinez-Martinez[3]

[1]*University of Guadalajara, Guadalajara, Mexico*
[2]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
[3]*Secretaria de Salud, Mexico City, Mexico*
maria.pita@cusur.udg.mx; pablolazareno@gmail.com; jose.alcazar@cusur.udg.mx; gervvan.bio@gmail.com; edderadonay@gmail.com; oscars49@hotmail.com

Keywords: Monocytes, HPV, Memory CD8+ T cells, CXR3CR1, cervical intraepithelial neoplastic-I

Introduction: Cancer is one of the diseases that has increased its incidence in recent years, particularly the numbers of new cervical cancer cases are increasing although this tumor is preventable. Some types of cancer are secondary to viral infections, in these cases the viruses usually induce the production of foreign proteins derived from your latent DNA. The Human Papillomavirus (HPV) has been identified as responsible for almost all cases of cervical cancer. The response of the immune system is crucial for tumor development, the changes occurring in the cervix mucosa and may have implication in development of different grades of Cervical Intraepithelial Neoplastic (CIN) lesions in cervical cancer. Monocytes plays an important role in the immune defense, inflammation, and homeostasis by the perception of their local environment, the clearance of pathogens and dead cells, and initiate adaptive immunity. Monocytes are classified in classical CD14+/CD16-, intermediate CD14+/CD16 + and non-classical CD14-/CD16+. It has been described that in mice loss of CX3CR1 increases accumulation of inflammatory monocytes.

A productive CD8+ T-cell response to a viral infection requires rapid division and proliferation of virus-specific CD8+ T cells. Moreover, CX3C receptor 1 (CX3CR1 seems to have an anti-inflammatory role by decreasing the production of chemokines and the infiltration of proinflammatory cells, preventing the immune response to cause inflammatory damage in the infected tissue. The expression of chemokine receptor CCR7 determines T cell exit from peripheral tissue to the lymph nodes and the expression of CD45RA on T cells is considered to be a marker of terminal differentiation and effector memory cell. On the other hand CX3CR1 distinguishes memory CD8+ T cells with cytotoxic effector function from those with proliferative capacity.

Aim: Characterize the proportions of monocytes and effector memory CD8+ T cells CXRCR1+ in peripheral blood of women with cervical intraepithelial neoplastic-I by flow cytometry.

Materials and Methods: A total of 20 women aged 25–45 years were enrolled in the study: 10 women without a diagnosis of cervical lesions (controls) by the Papanicolaou (PAP) test and 10 women with a diagnosis of CIN-I by biopsy. A blood sample was obtained by venipuncture in a tube with EDTA anticoagulant, in a first tube for cytometry was added 50 µL of peripheral blood and 5 µL of each of the following antibodies for lymphocytes: Anti-Human CD45RA FITC, Phycoerythrin (PE) anti-human CCR7, CD8a PerCP-Cyanine5.5 (eBioscience), APC anti-human CX3CR1 (Biolegend) Antibody. In a second tube for cytometry was added 50 µL of peripheral blood and 5 µL of each of the following antibodies for monocytes: Alexa Fluor 488 anti-human CD86 Antibody, PE anti-human CD16 Antibody, PerCP anti-human CD14 Antibody, APC anti-human CX3CR1 Antibody. The cell were analyzed in a FACS Calibur flow cytometer (BD Biosciences). Data were processed with the CELL Quest Pro software

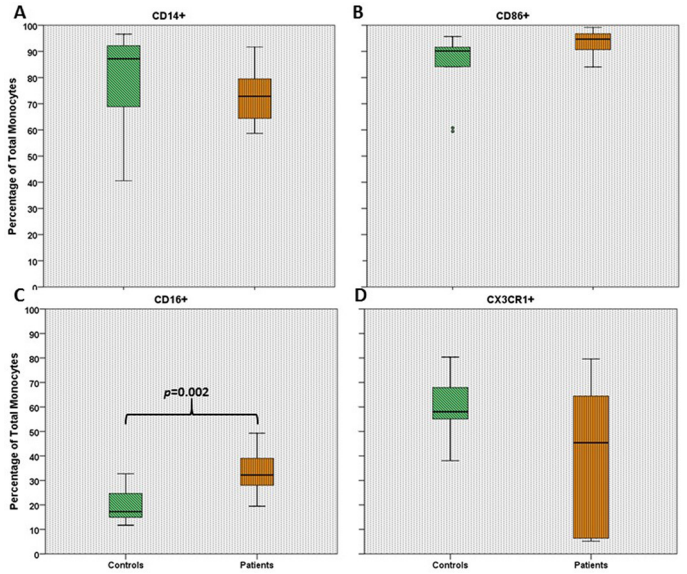


FIGURE 1: Percentages of peripheral blood total monocytes of from women with CIN-I (patients) and controls to markers CD14 (A), CD86 (B), CD16 (C) and CX3CR1 (D).

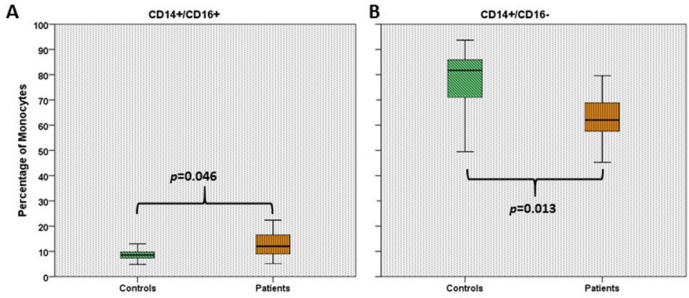


FIGURE 2: Percentages of peripheral blood intermediate monocytes CD14+/CD16+ (A) and classical monocytes CD14+/CD16- (B) of from patients and controls.

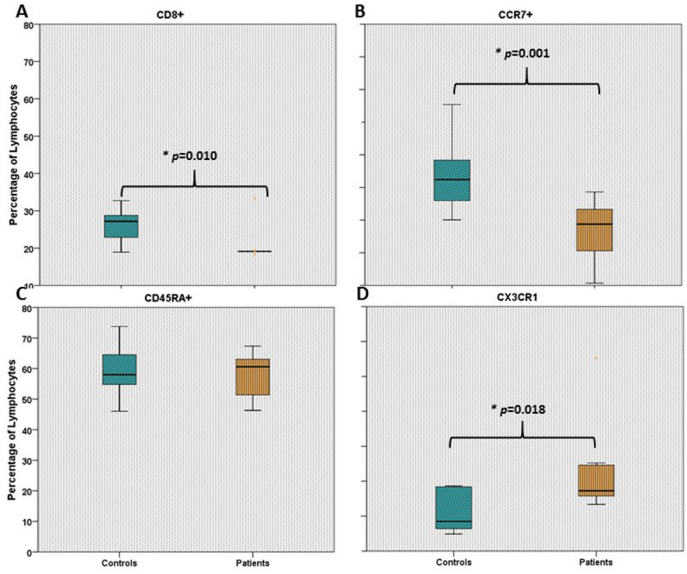


FIGURE 3: Percentages of peripheral blood T cells from women with CIN-I and controls to markers CD8 (A). CCR7 (B). CD45RA (C) v CX3CRl (D).

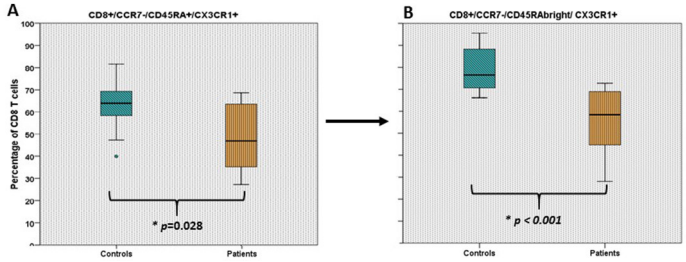


FIGURE 4: Percentages of peripheral blood T cells CD8+/CCR7-/CD45RA+/CX3CR1+ (A) and CD8+/CCR7-/CD45RA^{bright}/CX3CR1+ (B) from women with CIN-I and controls.

package (BD Biosciences) and the results are reported as percentages of expression. The results were analyzed in the software SPSS 22 and a statistical test of t was made.

Results: In monocytes CD16 showed a higher density in patients (33.72 %) than in controls (20.32 %), which was statistically significant (p = 0.02); the expression of CX3CR1 did not show statistically significant differences (p = 0.080) (Figure 1). The proportion of CD14+/CD16+ intermediate monocytes was higher in patients with 12.37 % compared to 8.49 % in controls (p = 0.046) and CD14+/CD16- classical monocytes was decreased (p = 0.013) in the group of patients (63.72 %) compared to controls (78.07 %) (Figure 2).

In the case of lymphocytes, the CCR7 marker showed a higher density in the controls (33.24 %) compared to the patients (16.75 %), showing a statistically significant difference (p = 0.001); the CD8 marker showed a similar pattern as its density was higher in the controls (26.13%) compared to the patients (20.47 %), showing statistically significant differences (p = 0.010), while the CX3CR1 was higher its density in patients (32.32 %) compared to controls (21.13 %), finding a statistically significant difference (p = 0.018) (Figure 3).

When analyzing the T lymphocyte subpopulations more thoroughly, the subpopulation CD8+/CCR7-/CD45RA+/CX3CR1+ was statistically significant different (p = 0.028), the density was higher in controls (62.68 %), compared to patients (47.79 %); from this subpopulation were selected the cells CD8+/CCR7-/CD45RA^{bright}/CX3CR1+ and were higher in controls (78.83 %) compared to patients (55.52 %) (p < 0.001) (Figure 4).

Discussion: In this study the group of patients showed a higher proportion of CD14+/CD16+ intermediate monocytes compared with controls, this subpopulation with proinflammatory characteristics and it is altered in several pathologies linked to chronic inflammation. The proportion of CD14+/CD16- classical monocytes was found to be decreased in the patients compared to the controls, some studies have suggested that this population of monocytes differentiates to CD14+/CD16+ under inflammatory conditions thus increasing the expression of CD16, this elevation can contribute to the defense against HPV infection.

The CCR7 is involved in the positioning of lymphocytes inside the secondary lymphoid organs and in the return of lymphocytes and dendritic cells to the line after passage through non-lymphoid tissues, aiding in migration and activation of T cells; It has been observed that activated T cells lose CCR7 on the cell surface, allowing them to enter microenvironments where they normally do not enter the inactivated cells, the low levels of CCR7 in peripheral blood T cells in patients could indicate that the T cells went through a process of migration and activation of T cells, thus losing the receptor on the cell surface. Ultimately, the coordinated expression of perforin, granulysin, granzyme has been associated with the expression of CX3CR1 and the differentiation of CD8 T cells effector memory, elevated levels of this receptor in patients may be involved in the differentiation of CD8 T cells effector memory in women with CIN-I.

Conclusions: Further immuno analysis are necessary to evaluate the accuracy of our preliminary results in order to complete the validation of frequencies of cells studied.

Transcriptional profile associated with tuberculosis-diabetes comorbidity in humans

Elena Jaime-Sánchez[1], Edgar Eduardo Lara-Ramirez[1], Juan Ernesto López-Ramos[1], Julio Enrique Castañeda Delgado[1], Yadira Bastian[1], Roberto Zenteno-Cuevas[2], José Antonio Enciso-Moreno[1]

[1]Unidad de Investigación Biomédica de Zacatecas (IMSS), Zacatecas, Mexico
[2]Universidad Veracruzana, Xalapa, Mexico
nena_smile@live.com; elarar0700@hotmail.com; ernesto.lopez92@hotmail.com; julioenrique_castaeda@yahoo.com.mx; yito21@yahoo.com; robzencue@gmail.com; enciso_2000@yahoo.com

Keywords: Comorbidity, Tuberculosis, immune response, type 2 diabetes, Molecular mechanisms, Metabolic pathways, Transcriptional profile

Diabetes mellitus is a risk factor to develop active tuberculosis (TB), mainly due to the alteration of the immune system. To date, little is known about the cellular and immune molecular mechanisms governing this co-morbidity. To uncover those processes, we performed a whole blood transcriptional analysis of tuberculosis patient groups with and without Type 2 Diabetes (TB-T2D and T2D groups), versus the healthy control group (HC). We found a transcriptome in the TB-T2D group which seems to be implicated in immune response and metabolic pathways. For example, IL-6R and SOCS3 mRNA transcripts were over-expressed only in TB-T2D group; we suggest this pathway may have an important role in the exacerbated inflammatory response to Mycobacterium tuberculosis (Mtb) in a T2D host, as the IL6R/SOCS3 pathways are involved IFN- inhibition signaling which is very important for tuberculosis control. Impairment in this cascade might have a direct effect on macrophage activation, compromising immune response against Mtb in the T2D host. Moreover, we also found PRKCD mRNA transcript is over-expressed in the TB-T2D. It is known that PRKCD activity blocks the rRNA transcription via TIF1A, a process linked to the decrease ATP level index of the cell. Besides, we found that some mRNAs for ribosomal subunit transcripts were down-regulated in TB-T2D group. Thus, it is possible that general protein transduction is impaired in TB-T2D patients, reinforcing there is a huge alteration in cell metabolism associated to this co-morbidity. Our results reveal some intriguing molecular mechanisms associated to TB-T2D co-morbidity.

Local immune response evaluation on UVB-immunosuppressed C57BL/6 mice infected with Leishmania mexicana

Angel Francisco González-Mireles[1], Mayra Alejandra Rodríguez-Serrato[1], Martha Maricela Solís-Treviño[1], Alma Yolanda Arce Mendoza[1], Angel Ramos-Ligonio[2], Mario César Salinas-Carmona[1], Alberto Yairh Limón-Flores[1]

[1]Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico
[2]Facultad de Ciencias Químicas, Universidad Veracruzana, Orizaba, Mexico
angel_gm7@hotmail.com; mayra_92_c@hotmail.com; marthasolis90@gmail.com; aya.mayola@yahoo.com; angramos@uv.mx; mario.salinas@uanl.mx; aylf10@hotmail.com

Keywords: Leishmaniasis, Cutaneous, Skin, immune suppression, UVB, Local immune response

Leishmaniasis is caused by a protozoa parasite from over 20 Leishmania species and is transmitted to humans by the bite of infected female phlebotomine sandflies. Cutaneous leishmaniasis is the most common form of leishmaniasis. There are several major risk factors associated with this disease, such as poverty, poor housing and domestic sanitary conditions, malnutrition, population mobility, and occupational exposure. Currently there is no scientific evidence that correlates the exposure to solar radiation (UVB) with the susceptibility to develop cutaneous leishmaniasis.

In this study, C57BL/6 mice were irradiated with several doses (1 to 15 kJ/m2) of UVB on shaved skin of the back using two sunlamps from Philips UVL (FS-40T12 UVB) and in vivo immune function was measured by delayed-type hypersensitivity (DTH) reaction. After the UV-induced immune suppression, mice were infected on the back with Leishmania mexicana mexicana promastigotes. We performed histopathological analysis and evaluated parasite burden and the presence of macrophages, mast cells, IL-10, and IFN-g. Results suggest a role of UVB in the susceptibility to develop cutaneous leishmaniasis.

Transcriptome analysis of two Mycobacterium tuberculosis Colombian clinical isolates from the LAM family reveals different virulence programs to cause pathogenesis

Andres Baena, Juan Pablo Isaza, Felipe Cabarcas, Juan Fernando Alzate, Luis Fernando Barrera

Universidad de Antioquía, Medellín, Colombia

andres.baenag@udea.edu.co; juanpabloisaza@gmail.com; felipe.cabarcas@udea.edu.co; jfernando.alzate@udea.edu.co; luis.barrera@udea.edu.co

Keywords: Mycobacterium tuberculosis, Virulence, Clinical Isolates, infectious diseases, RNAseq/transcriptome

Tuberculosis (TB) is a complex disease with a heterogeneous pathology and is caused by Mycobacterium tuberculosis (Mtb), which remains as the leading bacterial killer worldwide causing 1.8 million deaths annually [1]. Colombia is a country with a median incidence of TB, but certain vulnerable populations such as HIV infected people, jail prisoners, homeless and Indian aborigines are at high risk, with incidences comparable to African and Asian countries. One of the major concern of TB today is the uncontrolled spread of the MDR and XDR strains. It has been shown that, in some parts of the world, the antibiotic resistance is associated to strains such as Beijing/East Asian lineage [2]. Another important aspect of some Mtb strains concerns changes in their virulence that could be associated with a more progressive disease or with more transmission in certain populations. These aspects of the bacteria support the identification of those Mtb strains that are circulating in a specific population or patient in order to be more effective in their elimination. The new era of genomics has given a more comprehensive and comparative view of the whole genome of thousands of Mtb strains from all lineages, and the presence of compensatory and new mutations that could alter the fitness, the resistance to the immune system, increased transmissibility, hyper-virulence and more rapid progression to disease after infection have been recently discovered. Here we analyze two Colombian clinical isolates of Mtb, UT205 and, UT127, that were collected in Medellin, Colombia during a large cohort study conducted by the "Centro Colombiano para la Investigación en Tuberculosis" (CCITB) during 2005 to 2009 [3]. The two of them belong to the LatinoAmerican-Mediterranean family (LAM09) of Mtb. UT205 was obtained from a household in which an incident case was reported while no incident cases were reported in the household from which the UT127 strain was recovered, although the household contacts showed evidence of infection as tested by an in-house IGRA [3]. A published report of our research

group showed that UT205 is more virulent that UT127. UT205 was associated with an increased cell death with membrane damage and lower production of cytokines such as TNFα and IL-6 in infected macrophages [4]. Whole genome sequencing analysis showed that UT205 has a mutation that was associated with a Beijing clade in the mcr11 gene encoding a small regulatory RNA. This small RNA was shown to be implicated in mycobacterial cell growth and bacterial pathogenesis. Another frameshift deletion was identified in the kdpD gene that was also associated with other Beijing strains. This mutation can lead to the formation of non-functional proteins KdpD and KdpE, a two-component system involved with rapid expansion and virulence [5]. One of the main differences between the two clinical isolates was that UT205 grew significantly better than UT127 in Sauton's media while they grew similarly in 7H9 media. Comparative transcriptome analysis of the two strains UT127 and UT205 grown in two different media (Sauton and 7H9) showed that one of the most interesting genes that showed a more differential expression was mcr16 (downregulated in UT205 -5,6 log2FC related to UT127). A gen-network analysis (STRING) of the transcriptomic data showed that UT205 has more activation of different lipid pathways than UT127, such as: mmpL8 operon used for the synthesis and secretion of SL-1 and PAT, the FadD5 operon that regulates the transport and degradation of lipids, the molybdenum pathway and some of the DosR genes that regulate the hypoxia response. In conclusion, whole genome and transcriptome sequencing of Mtb strains isolated from patients with pulmonary TB allowed the identification of new genetic programs and profiles that might be associated with bacterial virulence and pathogenicity. These genetic programs may allow us to design personalized treatments in the future that could be more effective in the treatment of TB.

REFERENCES

1. WHO. Global Tuberculosis Report. 2017.
2. Periwal V, Patowary A, Vellarikkal SK, Gupta A, Singh M, Mittal A, et al. Comparative whole-genome analysis of clinical isolates reveals characteristic architecture of Mycobacterium tuberculosis pangenome. PLoS One 2015; 10(4):e0122979.
3. del Corral H, Paris SC, Marin ND, Marin DM, Lopez L, Henao HM, et al. IFNgamma response to Mycobacterium tuberculosis, risk of infection and disease in household contacts of tuberculosis patients in Colombia. PLoS One 2009; 4(12):e8257.
4. Duque C, Arroyo L, Ortega H, Montufar F, Ortiz B, Rojas M, et al. Different responses of human mononuclear phagocyte populations to Mycobacterium tuberculosis. Tuberculosis (Edinb) 2014; 94(2):111-122.
5. Freeman ZN, Dorus S, Waterfield NR. The KdpD/KdpE two-component system: integrating K(+) homeostasis and virulence. PLoS Pathog 2013; 9(3):e1003201.

The human cytomegalovirus UL8 glycoprotein reduces pro-inflammatory cytokine production by myeloid cells through its immunoglobulin-like domain

Pablo Engel[1], Pablo Martínez[1], Natalia Pérez-Carmona[1], Domènec Farré[1], Martin Messerle[2], Ana Angulo[1]

[1]Universitat de Barcelona, Barcelona, Spain
[2]Hannover Medical School, Hanover, Germany
pengel@ub.edu; pablomvi21@gmail.com; natalia.perez.carmona@gmail.com; dombcn@gmail.com; messerle.martin@mh-hannover.de; aangulo@ub.edu

Keywords: Cytokines, Myeloid Cells, Human Cytomegalovirus, immune evasion by microbes, Immunoglobulin superfamily

Human cytomegalovirus (HCMV) is a major pathogen that leads to life-threatening disease in immunologically suppressed and immunodeficient patients. Persistence of HCMV in infected individuals relies on a multitude of strategies to counteract host immune surveillance. To that end, HCMV devotes a wide repertoire of gene products, some of which have not been identified yet. Here we characterized the HCMV UL8 gene, which encodes a transmembrane protein with an N-terminal immunoglobulin (Ig)-like domain, an extended stalk, and a cytoplasmic tail bearing two potential tyrosine-based internalization motifs. The UL8 open reading frame gives rise to an extensively glycosylated protein, predominantly located at the cell surface, from where it can be partially endocytosed. By virtue of its highly conserved Ig-like domain, this viral protein selectively interacted with a surface molecule present on myeloid cells. Through site-directed mutagenesis, UL8 amino acid residues critical for ligand binding has been dissected. Notably, when ectopically expressed in THP-1 myeloid cells, UL8 was capable to substantially diminish the production of a large number of pro-inflammatory cytokines. Alteration of structural elements of UL8 indicated that this functional effect was mediated by the cell surface expression of its Ig-like domain. To assess the biological impact of the viral protein in the infection context, we constructed HCMVs missing the UL8 gene, and demonstrated that UL8 reduces the release of a variety of pro-inflammatory factors at late times after infection of THP-1 cells. Our findings indicate that UL8 may exert a subversive key role in host immune control, contributing to HCMV persistence.

Influence of Pseudomonas aeruginosa proteins OprF and aTox on citokine profile of mice

Nelli K. Akhmatova[1], Olga V. Lebedinskaya[2], E. O. Kalinichenko[1], Elina A. Akhmatova[1], Natalia A. Mikhailova[1]

[1]Research Institute of Vaccines and Sera. Mechnikov, Russian Academy of Medical Sciences (RAS), Moscow, Russia

[2]Perm State Medical Academy, Perm, Russia

anelly@mail.ru; lebedinska@mail.ru; gladius.domini@gmail.com; ela.150@yandex.ru; n_michailova@inbox.ru

Keywords: Mice, Pseudomonas aeruginosa, citokine, OPRF, ATOX

Introduction: Currently, infections caused by Pseudomonas aeruginosa are an important medical problem. Their treatment is extremely difficult because of the broad antibiotic resistance of the pathogen, so the issue of infection prevention, especially in hospitalized patients and patients with cystic fibrosis, becomes topical. In I.I. Mechnikov research Institutr (Moscow) is developing a candidate vaccine against Pseudomonas aeruginosa based on its recombinant proteins OprF and aTox (deletionary atoxic form of exotoxin A) sorbed on aluminum hydroxide.

Objective: to study the effect of immunization with the vaccine preparation against Pseudomonas aeruginosa on the concentration of cytokines in the blood serum of mice.

Materials and Methods: Preparation: 25 μg of OprF, 50 μg of aTox sorbed by 75 μg of aluminum hydroxide. For immunization, the recombinant protein preparation was mixed in equal weight fractions with an aluminum hydroxide gel, diluted in phosphate buffered saline (PBS), and sorbed for 12 hours at 4 ° C. The vaccine preparation was administered intraperitoneally in 0.5 ml to BALB/c mice.

Cytokine levels in sera and supernatants of spleen of mice were determined on a flow cytometer FC-500 (Beckman Coulter, USA) using a FlowCytomix Mouse Th1 / Th2 10 plex test system using beads sensitized with monoclonal antibodies to cytokines (GM-CSF, INF-γ , IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, TNF-α), eBioscience (USA).

Results: In 4 h, 8 h, 24 h and 14 days after immunization with a complex of recombinant OprF and aTox mice were used for total blood sampling.

The complex of recombinant OprF and anatoxin statistically significantly increased the levels of Th1/Th2/Th17/Th21/Th22 cytokines in sera at different times after administration: after 4 h, IL-1α, IL-5 (5.14; 1.9 times); 8 h later, IL-1β, IL-2, IL-4 (respectively, 13, 2.7, 1.4 times), and Th17/Th21/Th22 cytokines - IL-17A and IL-22 (respectively, 248 And 3.24 times) and IL-21 (from undetectable values to 17.13±1.59 pg/ml); after 24 hours, IL-6, IL-10, IL-12p70, TNF-a and IFN-γ, respectively (8.5, 10.8, 5.3, 3.7, 5.4 times).

Conclusion: 4 h after administration the antigenic preparation caused a significant increase in the secretion of IL-1α, IL-1β and TNFα cytokines, stimulating the synthesis of acute inflammatory phase factors, proliferation of lymphocytes and blood leukocytosis [Dolence J. et al., 2017]. TNFα is a multifunctional cytokine with effects not only in the proinflammatory response [Beutler B., 1995] but in immunoregulatory responses, and apoptosis [Wen H. et al., 2017]. TNFα is produced in numerous cell types and is initially synthesized as a transmembrane precursor that undergoes proteolytic cleavage from the cell surface to a soluble monomer of 17 kDa [Hehlgans T, Mannel DN. 2005]. 4 h after administration OprF/aTox caused a significant increase in the secretion of IL-2, directing the immune response through the Th1 pathway [Fu SH. Et al., 2017]; At the same time, the synthesis of IL-5 inducing a Th2-immune response and stimulating differentiation of B-lymphocytes increased more significantly. After 8 h, the levels of the mediators of the acute phase (IL-1β and TNFα), IL-2, IL-4 (causing the differentiation of Th0-lymphocytes into Th2) and IL-6 stimulating maturation of B-lymphocytes in plasmocytes continued to grow [Frey N. 2017].

In 24 h the immune response was represented by a significant increase in TNFα; IFNγ, IL-12 and IL-2, indicating the activation of the Th1-immune response. At the same time, the levels of IL-6 and the stimulator of the Th2-response, IL-10, increased. There was also a stimulating effect of the proteins on the levels of IL-17A, IL-21 and IL-22, responsible for the differentiation of Th17 cells, which play an important role in the activation of innate immunity and immune defense of mucous membranes [Saresella M. et al., 2011].

Thus, the candidate Pseudomonas aeruginosa vaccine based on its recombinant proteins OprF and aTox activates both the cellular and humoral parts of the immune system with the induction of the Th1 or Th2 response.

REFERENCES

Dolence JJ, Kobayashi T, Iijima K, Krempski J, Drake LY, Dent AL, Kita H. Airway exposure initiates peanut allergy by involving the IL-1 pathway and T follicular helper cells in mice. J Allergy Clin Immunol. 2017 Dec 14. pii: S0091-6749(17)31896-1. doi: 10.1016/j.jaci.2017.11.020.

Beutler B. TNF, immunity and inflammatory disease: lessons of the past decade. J Investig Med. 1995;43:227–235.

Wen H, Liu M, Liu Z, Yang X, Liu X, Ni M, Dong M, Luan X, Yuan Y, Xu X, Lu H. PEDF improves atherosclerotic plaque stability by inhibiting macrophage inflammation response. Int J Cardiol. 2017 May 15;235:37-41. doi: 10.1016/j.ijcard.2017.02.102. Epub 2017 Feb 24. PMID: 28262343

Baker SJ, Reddy EP. Modulation of life and death by the TNF receptor superfamily. Oncogene. 1998;17:3261–3270. doi: 10.1038/sj.onc.1202568.

Hehlgans T, Mannel DN. The TNF-TNF Receptor System. Biol Chem. 2002;383:1581–1585.

Fu SH, Yeh LT, Chu CC, Yen BL, Sytwu HK.New insights into Blimp-1 in T lymphocytes: a divergent regulator of cell destiny and effector function. J Biomed Sci. 2017 Jul 21;24(1):49. doi: 10.1186/s12929-017-0354-8.

Frey N. Cytokine release syndrome: Who is at risk and how to treat. Best Pract Res Clin Haematol. 2017 Dec;30(4):336-340. doi: 10.1016/j.beha.2017.09.002.

Saresella M, Calabrese E, Marventano I, Piancone F, Gatti A, Alberoni M, Nemni R, Clerici M. Increased activity of Th-17 and Th-9 lymphocytes and a skewing of the post-thymic differentiation pathway are seen in Alzheimer's disease. Brain Behav Immun. 2011 Mar;25(3):539-47. doi: 10.1016/j.bbi.2010.12.004.

Platelets promote B. abortus monocyte invasion by establishing complexes with monocytes

Aldana Trotta[1], Lis Noelia Velásquez[1], M. Ayelén Milillo[1], M. Victoria Delpino[2], Ana Maria Rodriguez[2], Verónica Inés Landoni[1], Guillermo Hernán Giambartolomei[2], Roberto Gabriel Pozner[1], Paula Barrionuevo[1]

[1]Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina

[2]Instituto de Inmunología, Genética y Metabolismo (INIGEM-CONICET), Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, Buenos Aires, Argentina

aldanatrotta@hotmail.com; lisnvelasquez@gmail.com; ayelen_milillo@hotmail.com; mdelpino@ffyb.uba.ar; anamrodriguez@gmail.com; vilandoni@yahoo.com.ar; ggiambart@ffyb.uba.ar; rpozner@hematologia.anm.edu.ar; pbarrion2004@yahoo.com.ar

Keywords: Brucella abortus, Brucellosis, platelets, early infection, monocytes/macrophages, complexes

Brucellosis is an infectious disease elicited by bacteria of the genus Brucella. Platelets have been extensively described as mediators of hemostasis and responsible for maintaining vascular integrity. Nevertheless, they have been recently involved in the modulation of innate and adaptive immune responses. Although many interactions have been described between B. abortus and monocytes/macrophages, the role of platelets during monocyte/macrophage infection by these bacteria remained unknown. The aim of this study was to investigate the role of platelets in the immune response against B. abortus. We first focused on the possible interactions between B. abortus and platelets. To assess this, human platelets were co-incubated with GFP–B. abortus for 4 h and the interaction was quantified by confocal microscopy and flow cytometry. Our results showed that platelets were able to interact with bacteria in a dose-dependent fashion. Moreover, this interaction triggered platelet activation, measured as fibrinogen binding and P-selectin expression ($p<0.001$). We further investigated whether platelets are involved in Brucella-mediated monocyte/macrophage early infection. To start evaluating this, THP-1 cells (monocytic human cell line) were infected with GFP–B. abortus (100:1) in presence or absence of platelets for 4 h and the effect of platelets on the infectious capacity of Brucella was analyzed by confocal microscopy. Our results demonstrated that the presence of platelets promote the invasion of monocytes/macrophages by B. abortus. Moreover, platelets establish complexes with infected monocytes/macrophages as a result of a carrier function elicited by platelets. The next aim was to further evaluate the ability of platelets to modulate functional aspects of monocytes in the context of the infection. First, we studied the secretion of immunomodulatory mediators. To address this, THP-1 cells were infected with B. abortus

in presence or absence of platelets for different time periods. The supernatants from infected cells were collected and quantified by ELISA. Next, we studied the expression of adhesion and co-stimulatory molecules on the monocyte surface by flow cytometry. The presence of platelets during monocyte infection enhanced IL-1β, TNF-α, IL-8 and MCP-1 secretion ($p<0.001$) while it inhibited the secretion of IL-10 ($p<0.01$). At the same time, platelets enhanced the expression of CD54 (ICAM-1) and CD40 ($p<0.01$). In order to investigate the mechanisms involved in their functional modulation, monocytes were stimulated with supernatants from B. abortus-infected platelets and surface CD54 was measured by flow cytometry. Our results showed that CD54 induction is, at least in part, due to soluble factors released by infected platelets ($p<0.001$). Furthermore, soluble factors released by B. abortus–activated platelets, such as sCD40L, PF4, PAF and thromboxane A2, are involved in CD54 induction. Overall, our results indicate that platelets can directly sense and react to B. abortus presence and modulate B. abortus-mediated infection of monocytes/macrophages increasing their pro-inflammatory capacity, which could promote the resolution of the infection.

Evaluation of serum biomarkers associated with tuberculosis

Juan Ernesto López[1], Eva Maria Salinas[2], Julio Enrique Castañeda[1], Yadira Bastian[1], Leonor Enciso[1], José Antonio Enciso[1]

[1]Unidad de Investigación Biomédica de Zacatecas (IMSS), Zacatecas, Mexico
[2]Autonomous University of Aguascalientes, Aguascalientes, Mexico
ernesto.lopez92@hotmail.com; emsalin@correo.uaa.mx; julioenrique_castaeda@yahoo.com.mx; yito21@yahoo.com; leonorenciso1@hotmail.com; enciso_2000@yahoo.com

Keywords: Proteins, Serum, Tuberculosis, biomarkers, diagnosis

Tuberculosis (TB) is a health problem that affects developing countries, causing millions of deaths per year. Diagnostic methods depend on the isolation and growth of the causative agent of the disease (Mycobacterium tuberculosis), which implies long diagnostic times (8 weeks). Currently, biomarkers of the disease capable of differentiating the active and latent forms of TB are analyzed and can also be integrated into rapid diagnostic tests. Based on the analysis of gene expression in individuals with different forms of TB, the search for serum markers that meet these characteristics can be initiated. Serum samples were obtained by peripheral blood centrifugation of patients

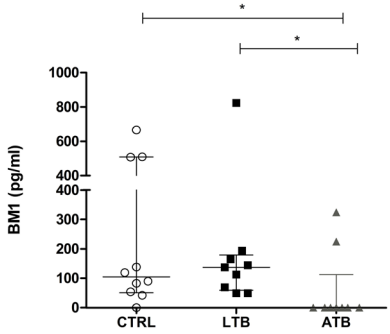


FIGURE 1: Levels of differential expression of biomarker 1 in serum samples from individuals with active tuberculosis (ATB), individuals with latent tuberculosis infection (LTB), and healthy individuals as control group (CTRL) n: 10. A Kruskal-Wallis test was performed, with a Dunn post-hoc to compare similarities between each pair of combinations of the groups (CTRL Vs LTB, CTRL Vs ATB and LTB Vs ATB) with a confidence interval of 95% ($p < 0.05$) (GraphPad Prism 5.0, CA., USA)

with Latent Tuberculosis (LTB), Active Tuberculosis (ATB) and healthy individuals as a control group (CTRL). The concentration of the protein "BM1" was evaluated in individuals of the three groups, by means of the commercial ELISA kit Peprotech® (USA), which contains a standard curve with which the concentration of the protein present in each serum sample (dilution of the sample 1: 5). A Kruskall-Wallis test was performed with Dunn's post-hoc to analyze the differences between the groups, with a confidence interval of 95%. Figure 1 shows how the protein called "BM1" was found to have low levels of concentration in serum samples from individuals that correspond to the group of patients with ATB, in comparison to those individuals belonging to the LTB and CTRL groups. According to the results obtained, a high concentration of the "BM1" protein in serum can be used as an indicator of absence of the active disease.

Some immunological features of ejaculate with asymptomatic bacteriospermia

Anatoliy P. Godovalov, Tamara I. Karpunina, Nikita V. Vavilov

Perm State Medical Academy, Perm, Russia
AGodovalov@gmail.com; karpuninapsma@mail.ru; nikione@yandex.ru

Keywords: Immunoglobulins, Inflammation, infertile couples, asymptomatic bacteriospermia, conditionally pathogenic microorganisms

Recently there has been a steady decline of the "norm" range in the spermogram indicators. The specific contribution of the male factor to the structure of infertility is 20–45% [5]. It is shown that men who are in infertile couples, often have asymptomatic bacteriospermia. It is described that microorganisms negatively affect the quality of the ejaculate, directly and indirectly [6]. It is known, the humoral factors of adaptive immunity are the components of protection against infection, therefore it is of interest to evaluate the content of immunoglobulins depending on the microbial spectrum of the ejaculate in asymptomatic bacteriospermia.

The aim of the study was to evaluate the content of immunoglobulins of M, G, A and E classes in the spermoplasm of men with asymptomatic bacteriospermia.

Materials and Methods: We made a laboratory study of ejaculate of 48 men who applied to the clinic for infertile couples, as well as 28 conditionally healthy volunteers. Tenfold dilutions of the starting material were prepared for the bacteriological analysis of the test samples. Bacteriological study was performed according to the generally accepted method [3, 4]. The total microbial number was expressed in lg CFU/ml. To establish the concentration of immunoglobulins (Ig) of class M (IgM), G (IgG), A (IgA) and E (IgE), the enzyme immunoassay method was used (Vektor-Best, Russia). The specific concentration of Ig was expressed in mg/g of total protein. The level of total protein was determined with the biuret reaction, and albumin level - with use of bromcresol green indicator (Vektor-Best, Russia). For statistical analysis, descriptive statistics methods, Student's t-test for unpaired data, $\chi 2$-criterion for qualitative characteristics were applied.

Results: All men who applied for infertile couples had microorganisms in the ejaculate. Of these, Gram-positive cocci (group 1; 4.53±0.24 lg CFU/ml) were found in 66.7% of men, and Gram-negative bacilli (group 2; 5.38±0.30 lg CFU / ml, $p<0.05$ to group 1).

Conditionally healthy volunteers (group 3) had no microorganisms' growth detected in the ejaculate. It was shown that in men of group 3 the protein concentration in the spermoplasma was 50.70±1.84 mg/ml, which is significantly lower than in group 1 (59.00±2.73 mg/ml, $p<0.05$) and group 2 (64.18±4.81 mg/ml, $p<0.05$). The quantity of albumin in the total protein fraction in group 2 was statistically significantly higher than in group 1 (Table 1). In patients of groups 1 and 2, the concentration of IgM and IgA was significantly reduced in the ejaculate, and IgG level was statistically significantly higher than in group 3 (Table 1). Immunoglobulins of E class were not found in any sample. In patients of groups 1 and 2, antisperm antibodies were detected in 31.6% of cases. The ejaculate of healthy volunteers had no such antibodies detected. The risk of formation of antisperm antibodies with asymptomatic bacteriospermia is 12.9 times higher than without it (95% CI 1.4–118.6). It is shown that morphological and motor characteristics of sperm cells change with asymptomatic bacteriospermia. Thus, the patients of groups 1 and 2 had a decrease in the total number of cells, the number of live and motile cells, and also cells with normal morphology (Table 2). As can be seen from Table 2, the presence of microorganisms can be associated with the ejaculate quality loss. In this case, the microflora is capable of adversely affecting both the number of cells and their functional activity.

Table 1: Protein, albumin and immunoglobulin content in the spermoplasm of men with asymptomatic bacteriospermia

Groups	Total protein, g/l	Albumin, g/l	Concentration of immunoglobulins, mg/g of protein		
			IgG	IgM	IgA
1 (n=32)	59.00±2.73$^{\#}$	21.12±1.41$^{*,\#}$	224.59±13.21$^{*,\#}$	4.58±0.95$^{\#}$	34.65±3.50$^{*,\#}$
2 (n=16)	64.18±4.81$^{\#}$	28.81±2.36$^{\#}$	275.14±18.31$^{\#}$	5.04±0.83$^{\#}$	24.53±2.10$^{\#}$
3 (n=28)	50.70±1.84	15.17±0.97	163.89±13.68	13.49±3.28	103.58±10.49

Note: * - $p<0.05$ when compared with the data of group 2, # - $p<0.05$ when compared with the data of group 3.

Table 2: Motor and morphological features of sperm cells of men with asymptomatic bacteriospermia

Groups	Total number of sperm cells, M/ml	Live sperm cells, %	Non-motile sperm cells, %	Average linear speed, μm/s	Sperm cells with normal morphology, %
1 (n=32)	67.99±14.97$^{\#}$	85.07±3.41$^{\#}$	39.82±7.41$^{\#}$	13.82±0.94$^{*,\#}$	57.07±4.51$^{\#}$
2 (n=16)	70.32±13.20$^{\#}$	89.60±3.89$^{\#}$	21.77±6.18$^{\#}$	10.25±1.28$^{\#}$	57.90±3.11$^{\#}$
3 (n=28)	110.32±10.16	99.00±0.01	3.58±0.25	16.55±0.45	64.10±0.68

Note: * - $p<0.05$ when compared with the data of group 2, # - $p<0.05$ when compared with the data of group 3.

Discussion: In the course of the conducted studies, it was shown that when persisting of representatives of Gram-positive and Gram-negative bacteria in the genital tract, a latent asymptomatic inflammation forms, which causes a persistent disruption of the permeability of the microcirculation [2], as a result of which transudation is intensified, but this mechanism requires further study. The microorganisms colonizing the genital tract have such properties that cells of the immune system do not provide an adequate response. This may be due to the fact that microflora produces a number of metabolites that exert a suppressor effect on immunocompetent cells. On the other hand, in the ejaculate with asymptomatic bacteriospermia, there is a slight decrease in the concentration of IgA and IgM, at which the existence and reproduction of conditionally pathogenic microorganisms is possible. In the present study, the ejaculate with bacteriospermia had increased IgG content, which, with a high probability, was a reflection of long standing inflammatory process. However, IgG activity is impaired and inadequate for resistance to conditionally pathogenic microorganisms, which may be due to the production of various radicals by microflora, and oxidized proteins are known to be practically incapable of performing their functions [1, 7]. In any case, the patients have a decrease in the number and motor performance of sperm cells, the increase in number of defective and nonviable forms increases, as well as in the production of antisperm antibodies. This situation requires not only antibiotic therapy, but also correction of the defect in the immune system.

Conclusion: Thus, despite the absence of a clinic picture, with asymptomatic bacteriospermia, there is an inflammatory process with disturbances in the microcirculation, as well as a change in the ratio of the main classes of immunoglobulins in the sperm. Against this background, there is a persistence of conditionally pathogenic microorganisms, which, having a number of advantages (metabolites with suppressor activity, synthesis of radicals) that ensure their prolonged stay in the genital tract, could negatively affect natural or artificial fertilization.

REFERENCES

1. Davies M.J. Protein oxidation and peroxidation // Biochem. J. 2016. 473(7). 805-25.
2. Elzanaty S., Erenpreiss J., Becker C. Seminal plasma albumin: origin and relation to the male reproductive parameters // Andrologia. 2007. 39(2). 60-5.
3. Godovalov A.P. Comprehensive assessment of microflora of intestine, vagina and hormonal levels in women with secondary infertility // Experimental & clinical gastroenterology (Russian). 2016. № 3 (127). C. 59.
4. Godovalov A.P., Karpunina T.I. Microbiological and morpho-functional features of ejaculate from infertile men with asymptomatic bacteriospermia // International Research Journal. 2016. № 9-3 (51). C. 34-38.
5. Kumar N., Singh A.K. Trends of male factor infertility, an important cause of infertility: A review of literature // J. Hum. Reprod. Sci. 2015. 8(4). 191-6.

6. Vilvanathan S., Kandasamy B., Jayachandran A.L., Sathiyanarayanan S., Tanjore Singaravelu V., Krishnamurthy V., Elangovan V. Bacteriospermia and Its Impact on Basic Semen Parameters among Infertile Men // Interdiscip. Perspect. Infect. Dis. 2016. 2016. 2614692.
7. Yan M., Zhang X., Pu Q., Huang T., Xie Q., Wang Y., Li J., Wang Y., Gu H., Huang T., Li Z., Gu J. Immunoglobulin G Expression in Human Sperm and Possible Functional Significance // Sci. Rep. 2016. 1(6). 20166.

Opportunistic diseases in HIV/aids patients with aids premiere that receiving antiretroviral therapy

Dayme Hernandez

Sociedad Cubana de Inmunlogía (SCI), Habana, Cuba
dayme@ipk.sld.cu

Keywords: Viral Load, hiv/aids, PCP, antiretroviral treatment, CD4+ T lymphocyte, Late diagnosis, Opportunistic diseases, Neurotoxoplasmosis, waste syndrome.

Introduction: The AIDS premiere is a form of presentation of the illness caused by HIV that is characterized by alteration of the patient's general state, waste syndrome, appearance of serious opportunists infections, neoplasia and neurological alterations. The antiretroviral treatment has increased the life expectancy of these patients.

Objectives: To identify the opportunistic diseases associated to the clinical condition under study and their linking to the CD4+ T lymphocyte count and the viral load as well as to evaluate the mortality in the studied group and its relationship with opportunistic diseases.

Methods: Prospective, observational and cross-sectional study of a sample of 55 patients. The size of the sample depended on the total universe of HIV/aids patients in the medicine service of "Pedro Kourí" Tropical Medicine Institute. The study took into account those subjects who were diagnosed with aids debut and presented with opportunistic diseases during one year.

Results: Infectious opportunistic diseases such as neurotoxoplasmosis (21.8 %) and pneumonia caused by Pneumocystis jirovecii (12.7 %) were the predominant defining events of aids. There was no statistically significant association with low CD4+ T lymphocyte count and high viral load. In patients over 50 years of age with more than one opportunistic disease, the risk of dying was 4.72 times higher than in the rest of the group.

Conclusions: Infectious opportunistic diseases as neurotoxoplasmosis and Pneumocystis jirovecii were the prevailing defining events of aids. Aids-associated

mortality in patients aged over 50 years increased in individuals who presented more than one opportunistic disease. These results are useful for the design of treatment strategies that reduce the occurrence of opportunistic diseases and improve even more the survival of HIV/aids patients.

The role of microRNAs in regulating autophagy and the inflammatory response during Mycobacterium tuberculosis infection

Joyoti Basu

Bose Institute, Kolkata, India
joyotibasu@gmail.com

Keywords: Cytokines, Inflammation, MicroRNAs, Mycobacterium tuberculosis, Autophagy.

Tuberculosis presents as a continuum of lesions of varying types due to the complex interplay between the bacterium and its host. We have analysed how differential regulation of miRNAs during M. tuberculosis (Mtb) infection, impacts the inflammatory response and autophagy. Global miRNA profiling and miRNA-mRNA network analysis performed by us provided the platform for initiating these investigations.

We asked the question how the miRNA–mRNA network in Mtb- infected macrophages regulates the immune response. We demonstrate a role of the TNFAIP3(A20)/let-7f signalling axis in regulating the inflammatory response. The let-7 miRNAs are highly connected nodes of the miRNA-mRNA network in infected macrophages. A20, a negative regulator of NF-kappa B signaling is a target of let-7f. Augmented release of TNF-alfa, IL-1beta, CCL2, CXCL1, IL-6 and NO occurs in Mtb-infected macrophages in the absence of A20, whereas transfection with a let-7f mimic, leads to increased cytokine release after infection. The early decrease in let-7f following infection, appears to offer an advantage to the pathogen as assessed by CFU counts in the absence or presence of let-7f mimic. The second miRNA of interest, miR17-5p, was downregulated in the early stages of infection of macrophages with Mtb. Overexpression of a miR17 mimic augmented autophagy in Mtb-infected macrophages. miR-17 was downregulated in infected mouse lungs. We validated Mcl-1 as a miR-17 target by establishing the inhibitory effect of a miR17 mimic on the activity of a luciferase reporter fused to the 3'UTR carrying the putative miR17 binding site. We demonstrated that forced expression of miR17, dampens the levels of Mcl-1 in infected macrophages. We hypothesized that during infection, the early downregulation of miR17, promotes upregulation of Mcl-1, thereby inhibiting autophagy and offering Mtb a survival advantage within the infected macrophage. On a similar note, we observed that the transcription factor KLF4 is transcriptionally downregulated, yet translationally upregulated, during the early hours of Mtb infection. We linked this apparent discord to the downregulation

of miR-126a which targets KLF4. KLF4 once again, targets autophagy by transcriptionally regulating Mcl-1, thereby inhibiting autophagy as well as the trafficking of Mtb to lysosomes. These studies highlight the potential of miRNA-based host-directed therapeutic approaches for the control of TB.

Characterizacion of the inhibitory effect induced by TGF-beta during in vitro cell infection by h1n1 influenza virus

José-Luis Montiel-Hernandez, Genoveva BustosRivera-Bahena, Judith González-Christen, Fernando R. Esquivel-Guadarrama, Vanessa López-Guerrero

Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
jlmontiel@uaem.mx; noge77@gmail.com; judith.gonzalez@uaem.mx; fernando.esquivel@uaem.mx; vannylpz@yahoo.com

Keywords: Cell Death, p38 Mitogen-Activated Protein Kinases, Influenza Virus, signalling pathways, TGF-beta, Smad 7

Flu is an acute respiratory disease caused by the infection of the influenza virus. Several studies suggest an association between the severity of the disease and the presence of a massive local inflammatory response, also known as "cytokine storm". Although, the TGF-beta is well-recognized as a key immunorregulatory factor, is poorly known its role during the influenza infection. Previous reports have suggested an inhibitory role of this cytokine in the course of in vitro and in vivo virus infection but the mechanisms remain unknown.

Objective: To characterize the inhibitory effects of TGF-beta during the in vitro infection by the influenza virus.

Material and Methods: In vitro cell infection was done employing the influenza virus H1N1 (New Caledonia/99) in MDCK and A549 cell lines. The cell infectivity was monitored by hemagglutination and lytic plaque-assays. The presence of NS1 or M1 and phosphorylation of Smads and p38 were done by western blot. Cell death determination and levels of I and II TGF-beta receptors were done by flow cytometry and western blot. Cell death pathways were evaluate by antibody microarray (R&D). IL-1beta, TNF and TGF-beta1 were determined by ELISA.

Results: In both cell systems, the infection by the influenza virus was confirmed by hemagglutination assay, lytic-plaque assay and expression of the viral protein NS1/M1. In our system, the maximum of cell death induced by virus infection was obtained at

24 hrs post-infection (56.5%). Two hours pre-incubation with TGF-beta1 induced a partial inhibition of the viral infection measured as cell death (26%) and expression of viral protein NS1 (58.8%). In these conditions, we not observed a change in the phosphorylation level of Smads 2/3, but the phosphorylation of p38 and expression of Smad7 were significantly increased, suggesting its participation. It was also observed a significant increase in the TGF-beta's receptors I and II, that it seems to match with an increase of TGF-beta in the culture medium and a reduction of IL-1beta and TNF.

Conclusion: Our results suggest an inhibitory role of TGF-beta in the course the influenza virus in vitro infection, where participation of p38 and Smad7 could be involved. Additionally, different cell death pathways and inflammatory markers were controlled by TGF-beta pre-treatment.

Dysfunctional IL-17-producing CD8+ T cells are associated with high immune activation in HIV-infected patients under HAART-induced viral suppression

Federico Perdomo-Celis[1], Natalia A. Taborda[1,2], Maria T. Rugeles[1]

[1]Universidad de Antioquía, Medellín, Colombia
[2]Corporación Universitaria Remington, Medellín, Colombia
federico.perdomo@usco.edu.co; natalia.taborda@uniremington.edu.co; maria.rugeles@udea.edu.co

Keywords: HIV, CD8 T cells, CD161, IL-17A, immune activation, HAART

Immune activation is the hallmark of human immunodeficiency virus 1 (HIV) infection, even in patients with viral control induced by highly active anti-retroviral therapy (HAART). A major cause of immune activation during this infection is the marked intestinal microbial translocation as a consequence, among other factors, of a decrease and/or dysfunction of interleukin 17A (IL-17)-producing T cells, as this cytokine promotes the integrity of the intestinal barrier. Although IL-17-producing CD4+ T cells, characterized by the expression of CD161, have been widely studied, a population of IL-17-producing CD8+ T cells has been also described. However, their relation with microbial translocation and immune activation in non-viremic HIV-infected patients on HAART is unclear. By flow cytometry, we characterized circulating CD161-expressing and polyclonally-stimulated IL-17-producing CD8+ T cells in HIV-infected patients under viral suppression by HAART (viral load <50 HIV RNA copies/mL; n=30). All patients were on their first therapeutic scheme and had not developed previous therapeutic failure. Seronegative individuals were also included as controls (n=9). The association of IL-17 production by CD8+ T cells with the expression of cellular and soluble activation markers was also evaluated. Circulating CD161-expressing CD8+ T cells were decreased in HIV-infected patients in comparison with seronegative individuals, although these cells exhibited a low activation state in both groups of individuals, evaluated by the expression of HLA-DR and CD38. Conversely, after a polyclonal stimulation with PMA/ionomycin, the frequency of total IL-17-producing CD8+ T cells in HIV-infected patients was similar to that of seronegative individuals. Among total CD8+ T cells, IL-17 production was enriched in those cells expressing HLA-DR and CD38 in seronegative individuals. Interestingly, in comparison with seronegative controls, HIV-infected patients had a higher frequency of HLA-DR+ CD38+ CD8+ T cells after polyclonal stimulation, but these cells had a lower production of IL-17. Finally, to determine the association of these alterations with the microbial

translocation and immune activation state in HIV-infected patients, we evaluated the levels of soluble CD14 (sCD14) in plasma; low production of IL-17 by HLA-DR+ CD38+ CD8+ T cells was correlated with high levels of sCD14 in these individuals. Our findings suggest that IL-17 production by CD8+ T cells is associated to an activated phenotype in healthy individuals and, despite HAART, in HIV-infected patients this population is dysfunctional. These alterations could contribute to the persistent microbial translocation and immune activation in treated HIV-infected patients. The IL-17-producing CD8+ T cells frequency could constitute a novel progression marker in non-viremic treated HIV-infected patients and a potential therapeutic target for the reconstitution of microbial translocation and immune activation.

Human Neutrophil response against the hyper-epidemic clone of carbapenem resistant Klebsiella pneumoniae ST258

Luis A. Castillo[1], Nahuel Rodriguez Rodrigues[1], Federico Birnberg-Weiss[1], Pablo Schierloh[1], Daiana Martire Greco[1], Sonia A. Gomez[2], Veronica Landoni[1], Gabriela Cristina Fernandez[1]

[1]Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina

[2]Instituto Nacional de Enfermedades Infecciosas Dr. Carlos G. Malbrán (INEI), Buenos Aires, Argentina

luiscastillo718@gmail.com; nahuelrodriguezr@gmail.com; federicobirnberg@hotmail.com; lp_shier@hotmail.com; daianamartire@hotmail.com; gomezs1015@yahoo.com.ar; vilandoni@yahoo.com.ar; gfernandez@hematologia.anm.edu.ar

Keywords: Klebsiella pneumoniae, Neutrophils, Evasion strategies, KPC, lipopolysaccharide (LPS)

Klebsiella pneumoniae (Kpn) has become a relevant nosocomial pathogen due to the acquisition of multiple antibiotic resistance genes, emerging as a serious public health problem. Kpn isolates classified as Sequence Type 258 (ST258) by multi-locus sequence typing are largely responsible for global spread of the blaKPC gene, which codifies for Kpn-Carbapanemase (KPC), an enzyme that mediates resistance to carbapenems, the last option in antibiotic treatment. In order to determine whether the rapid dissemination and persistence of Kpn-KPC ST258 could be related to mechanisms of immune evasion, in this study we evaluated if Kpn-KPC induced a differential response in neutrophils (PMN), compared to another opportunistic pathogen.

For this purpose, we evaluated different parameters of activation in human isolated PMN challenged with a local clinical isolate of Kpn-KPC ST258 or Eschericia coli ATCC 25922 (Eco), using a multiplicity of infection (MOI) of 20. CD11b expression was measured using an anti-CD11b antibody, and reactive oxygen species (ROS) using dihydrorhodamine 123 (DHR) by flow cytometry, after 30 min. of stimulation with the different bacteria. Neutrophil extracellular traps (NETs) formation was evaluated after 3 hours of challenge by confocal microscopy using a DNA and elastase staining and by quantification of the NETs-released myeloperoxidase (MPO) (using TMB substrate) and DNA (using Pico-Green) after DNase digestion. Although up-regulation of CD11b expression was similar for both bacteria, NETosis and ROS generation were lower in the presence of Kpn-KPC compared to Eco (NETs area, mm2x10-4: Kpn-KPC=0.22±0.06; Eco=2.17±0.22, $p < 0.05$; MPO, Arbitrary Units: Kpn-KPC=0.09±0.03; Eco=0.70±0.10, $p<0.01$; DNA, ng/mL: Kpn-KPC=299.1±34.0; Eco= 631.4±77.1,

p<0.05); ROS generation (% DHR+ PMN: Kpn-KPC=5.2±0.1; Eco= 39.8±11.2, p< 0.01). Moreover, PMN chemotactic migration, evaluated by a Boyden chamber, was also diminished in Kpn-KPC-challenged PMN compared to Eco (Number of PMN migrated: Kpn-KPC= 25±2; Eco= 45±4, p<0.05).

In order to determine whether the decreased induction of bactericidal mechanisms by Kpn-KPC (ROS and NETs induction) was associated to a higher bacterial survival, we evaluated the surviving colony forming units after 1 hour of incubation with PMN. As expected, we observed that Kpn-KPC was more resistant to PMN-mediated killing compared to Eco (% Bacterial Survival: Kpn-KPC= 88.8±4.3; Eco= 31.3±7.0, p<0.05). When we evaluated phagocytosis by flow cytometry, using bacteria stained with FICT, no differences were observed in the percentage of phagocyted Kpn-KPC or Eco.

Next, we decide to focus our study investigating the mechanisms involved in the inhibition of ROS generation by Kpn-KPC. In this sense, we first asked if the lower induction of ROS by Kpn-KPC depends on a membrane component. To elucidate this, a Kpn-KPC bacterial extract (KpnBE) enriched in bacterial cell-wall molecules was performed using a mechanical disruption protocol. We found that KpnBE was able to reduce ROS induced by two different seven-transmembrane receptor agonists, fMLP 1μM (% DHR+ PMN: fML*P*=70.2±2.1; KpnBE+fML*P*= 21.9±3.1, p< 0.01) and Leukotiene B4 (LB4) 100 ng/mL (% DHR+ PMN: LB4=41.4±4.1; KpnBE+LB4=23.9±5.2, p<0.05). Moreover, KpnBE affected IL-8 production induced by fMLP in PMN (IL-8 ng/mL: fML*P*=1143±179; KpnBE+fML*P*=531±130, $p < 0.05$). As calcium is an important intermediate in the seven-transmembrane receptor signaling, we evaluated if KpnBE was able to modulate intracellular Ca2+ mobilization induced by fMLP, using a Fluo-3AM probe by flow cytometry. No differences were observed in Ca2+ mobilization between fMLP and fMLP+KpnBE. This result indicates that interaction fMLP-receptor may not be affected by KpnBE, and that the inhibition of ROS may be independent of Ca2+ mobilization or may involve a down-stream mechanism.

To elucidate which bacterial component was mediating the inhibition of ROS in PMN, LPS lipid-neutralization by Polymyxin B (PB) or heat inactivation of KpnBE were performed. None of these treatments reversed the KpnEB's inhibitory activity. Moreover, KpnBE-precipitated proteins did not induce per se any ROS inhibition, indicating that no protein component was involved. On the other hand, when KpnBE was treated with Periodic Acid (PA) to oxide polysaccharides in order to inactivate their biological function, this oxidized KpnBE lost its inhibitory properties of fMPL-induced ROS production (% DHR+ PMN: fML*P*=64.5±9; oxidized-KpnBE+fML*P*=51.5±8). In line with this finding, depletion of mannose containing molecules in KpnBE performed by Concanavalin A (ConA) treatment was not

able to reduce ROS induced by fMLP (% DHR+ PMN: fML*P*=66.5±4; ConA treated-KpnBE+fML*P*=63±11). These results indicate that a saccharide is involved in ROS inhibition.

Although the lipid part of LPS was proven not to be involved in the observed inhibition of ROS, considering the above results and the fact that LPS also contains an important saccharide component, we used a Polymyxin B-agarose column to deplete the entire molecule of LPS from KpnBE, and found that these depleted extracts were no longer inhibitory. Conversely, the column-retained LPS (Kpn-KPC-LPS) were eluted using sodium deoxycolate and tested for their inhibitory activity. Kpn-KPC-LPS (100 ng/ml) impaired ROS production induced by fMLP (% DHR+ PMN: fML*P*=60±2; Kpn-KPC-LPS+fML*P*=43±3, $p< 0.01$).

Our results revealed that Kpn-KPC ST258 showed a survival advantage compared to other enterobacteria, like Eco, by poorly triggering PMN responses (NETs, ROS and chemotaxis). Furthermore, we proved that a membrane component of KpnBE inhibited ROS and IL-8 induced by fMLP, effects that were independent of a deranged Ca2+ mobilization. Finally, we demonstrated that the polysaccharide component of Kpn-LPS was, in part, responsible of the inhibition of ROS. In conclusion, our results suggest that Kpn possess bactericidal evasion mechanisms that could be a potential advantage for the dissemination/persistence of Kpn infections.

Murine model of early neonatal invasive candidosis

Orlando Esau Flores Maldonado, Luis Antonio Rodriguez Gamez, Romel Hernández Bello, Gloria González González, Miguel Ángel Becerril García

Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico
orlandofloma10@gmail.com; luisrdzg_6@hotmail.com; romelhdezb.d@gmail.com; orlando_rko@hotmail.com; migb16@yahoo.com.mx

Keywords: Candida albicans, Patogénesis, Recién nacido, Candidosis diseminada, Modelo murino

Introduction: Invasive neonatal candidosis is an important cause of morbidity and mortality in neonates, especially in premature newborn. The pathogenesis during invasive candidosis has been widely studied in humans and adult experimental models, however, very little is known about the pathophysiology and mechanisms of the immune response that are established during this infection in the neonatal period.

Aim: Establish a 24-hours-old neonatal invasive candidiasis murine model.

Material and Methods: BALB/c mice less than 24-hours-old and females 6–7 weeks old were infected intravenously with 1x105, 5x105, 1x106 and 1x107CFUs of Candida albicans ATCC 660027 to evaluate survival. On the other hand, newborn and adult mice were infected with 5x105y 1x106CFU of C. albicans respectively, the animals were sacrificed on days 1, 2, 3, 4 and 6 after infection. Brain, kidney, lung, liver and spleen were harvested and immediately homogenized for colony counts; additionally, we perform a histological analysis of the tissues by staining Periodic acid–Schiff.

Results: Newborn animals die at early times of infection using an equal or lower amount of yeast than adultsand, like them, survival depends on inoculum yeast concentration. Interestingly, the pups infected with 1x105CFU (amount adjusted according to the weight of the newborns) have a survival of 100% at day 6 after infection. While in adults the kidney is the main organ affected, in newborns the liver is the tissue with the highest fungal burden and invasion of yeast and filaments for 1x105 and 5x105 of yeast inoculated.

The fungal burden and the histopathological aspect of the kidney and lung of the pups infected with 1x105 and 5x105remain unchanged and were the organs least affected

during the infection. However, the liver, spleen and brain of newborns infected with 1x105 showed a CFUs reduction and less tissue damage at day 3 post-infection,unlike at the same organs of pups infected with 5x105 that increase in fungal burden and invasion of fungal structures.

Conclusions: We have established a murine model of invasive neonatal candidosis using mice less than 24-hours-old. We show that newborn animals die at early times compared with adults; however, adjusting the inoculum to the newborns weight, pups present a high percentage of survival. The liver is mainly affected in neonates. The fungal burden and the histopathological damage of the sites of dissemination was different among them, likewise when compared neonates and adults. The development of this model will allow to deepen about the neonatal invasive candidosis immunopathogenesis, as well as the search for biomarkers for the diagnosis and prognosis of the disease during the neonatal period.

Role of bovine lactoferrin in the cellular and humoral immune response during the acute phase of infection caused by Trypanosoma cruzi in BALB / c mice

Edgar Jair Rodriguez, Edgar Marin, Ivonne Arciniega, Eder Jonathan Fernando Coronel, Emma Yael Gutierrez, Rafael Campos, Aldo Resendiz Albor

Escuela Superior de Medicina (IPN), Mexico City, Mexico
henry_2330@hotmail.com; edmarsur@yahoo.com.mx; ivonne.arciniega.77@gmail.com; coronel_ayala@outlook.com; emma.gtz.a@hotmail.com; citlicampos@gmail.com; alrealdo@yahoo.com.mx

Keywords: Flow Cytometry, Lactoferrin, Trypanosoma cruzi, ELISA assay, Inmune response

Trypanosoma cruzi, is the protozoan parasite that causes American trypanosomiasis or Chagas disease, which affects 8 million people and around 65 million are at risk of infection throughout the Americas. T. cruzi infection presents an acute phase followed by an asymptomatic chronic called indeterminate. Currently, two drugs are used in the treatment of the disease: Benznidazol and Nifurtimox, however, have a very limited efficiency in chronic patients which has limited their use. Therefore, the lack of an efficient drug for its treatment has required the development of new strategies to treat this disease. INC-5 is a strain that was isolated in the year of 1997 from a human clinical case from the state of Guanajuato. Previously it has been demonstrated in an infection model, that bovine lactoferrin reduces the parasitic load by 50% and increases the survival of BALB / c mice, so in this work the immunomodulatory role of bovine lactoferrin in mice was evaluated against T. cruzi. BALB / c mice were immunized and subsequently challenged with inoculum of 1x102 blood trypomastigotes intraperitoneally. Parasitemia was counted at 5, 7, 11, 14, 17 and 21 days post infection using the Pizzi-Brener method. Cytokines (TNF-α, IL-12, INF-γ, IL-4, IL-5, IL-6) were determined by intracellular stains subsequently analyzed by flow cytometry and antibody levels were measured from the serum sample. IgM, IgG, IgG1 and IgG2a using the ELISA technique. The administration of lactoferrin induces the production of IgG1, IgG2a antibodies which participate in the protection of mice infected with the parasite. The increase in the populations of cytokine-producing lymphocytes TNF-α, IL-12, INF-γ, IL-4, IL-5, IL-6 was observed. These data together indicate that bovine lactoferrin induces a protective immune response during the acute phase of infection that involved mainly the production of IgM and IgG antibodies and subclasses IgG1

and IgG2a, corresponding to a profile of Th1 and Th2 cytokines which was checked by flow cytometry in splenic cells with a high production of TNF-α, IL-12 and INF-γ and a Th2-type response with the increase of IL-6 and IL-4 which participate in the protection against Trypanosoma cruzi.

Central Nervous System inflammation due to the respiratory syncytial virus infection

Karen Bohmwald[1], Janyra A Espinoza[1], Jorge Soto[1], María Cecilia Opazo[2], Ayleen Fernández[1], Valeria Erendira Mateo-Estrada[3], Eliseo A Eugenin[4], Claudia A Riedel[2], Alexis M Kalergis[1,5]

[1]Pontificia Universidad Católica de Chile, Santiago, Chile
[2]Universidad Andrés Bello, Santiago, Chile
[3]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[4]Rutgers New Jersey Medical School, Rutgers University, Newark, United States
[5]INSERM UMR1064 Centre de Recherche en Transplantation et Immunologie, Nantes, France
nerakzita@gmail.com; janyra.espinoza@gmail.com; jorge.sotoramirez@gmail.com; cecilia.opazo@gmail.com; ayleen.fernandez@gmail.com; vmateo@lcg.unam.mx; eugeniea@njms.rutgers.edu; criedel99@yahoo.com; akalergis@bio.puc.cl

Keywords: Astrocytes, Central Nervous System, Inflammation, respiratory syncytial virus, Behavior alteration.

The human Respiratory Syncytial Virus (hRSV) is the major cause of lower respiratory tract illness in infants and young children worldwide, including severe bronchiolitis and pneumonia. Recently, neurologic alterations have been associated with hRSV infection in children, which include seizures, central apnea and encephalopathy. Further, hRSV impairs behavioral and learning processes in animals probably due to either an altered production of pro-inflammatory cytokines or by the CNS cells infection. First, we evaluated if the hRSV infection promotes the immune cell transmigration into the CNS and we observed that neutrophils, resident macrophages and inflammatory monocytes are increased at day 3 post-infection, whereas B lymphocytes and CD8 + T cells are increased at 7 days post-infection, Then, we analyzed the pattern of cytokine expression by RT-qPCR and ELISA in the brain of mice intranasally challenged with of hRSV, observing a down regulation of CD200 and an elevated IL-6, TNFα and IL-4 expression levels. hRSV-infected murine astrocytes showed an increased production of nitric oxide (NO), GFAP, IL-6 and TNFα, suggesting an activation of astrocytes and a pro-inflammatory profile in the CNS. Furthermore, similar results were found using hRSV and human astrocyte cultures for viral loads and GFAP levels. Moreover, hRSV infection caused an acute and chronic behavior impairment (up to two months post infection). In these mice we found, an altered expression of cytokines such IL-4, IL-10 and CCl2, as well as increased GFAP levels in mice with a severe behavioral impairment due to hRSV infection. This work suggests that hRSV-infection can impair the proper CNS function due to local inflammation and that astrocytes can be targeted during this process.

IL-10 production by Myeloid derived suppressor cells during Klebsiella pneumoniae ST258 infection induces an anti-inflammatory environment required for host survival

Hernan Felipe Penaloza[1,2], Loreani P Noguera[1,2], Francisco Javier Salazar[1,2], Omar P Vallejos[1,2], Liliana González[1,2], Isidora Suazo[1,2], Susan M Bueno[1,2]

[1]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile., Santiago, Chile
[2]Millennium Institute on Immunology and Immunotherapy (MIII), Santiago, Chile
hfpenalo@gmail.com; lpnoguera@uc.cl; salazar.echegarai@gmail.com; opvallejos@uc.cl; gonzalezandrea223@gmail.com; idsuazo@uc.cl; sbueno@bio.puc.cl

Keywords: Interleukin-10, Lung damage, carbapenem-resistant Klebsiella pneumoniae, Immune response modulation, Monocytic myeloid derived suppressor cells

Carbapenem-resistant K. pneumoniae sequence type 258 (CRKPN-ST258) are a major concern of public health worldwide. These pathogens not only have acquired antimicrobial resistance, but also strategies to evade the host immune response. Using an IL-10/GFP transgenic mice infected with a highly prevalent strain of CRKPN-ST258 strain (KP35), isolated from New York City, we identified that during KP35 infection, there is a rapid and consistent recruitment of Monocytic Myeloid-Derived Suppressor cells (M-MDSCs) to the lung tissue. These cells were able to produce Interleukin-10 (IL-10) from the first 48 hours post-infection and up to 96 hours post infection. Next, we evaluated whether IL-10 produced by M-MDSCs is a main suppressive mechanism of the immune response during KP35 infection. Using an IL-10-/- mice, we have found that these mice presented an higher susceptibility to KP35 infection, accompanied with elevated bacterial burden in the airways and increased lung tissue damage. Importantly, transfer of Bone marrow MDSCs (BM-MDSCs) to IL-10-/- receptor mice decreases their mortality, improves their bactericidal capacity in the lung tissue and prevented the infiltration of neutrophils to the lung tissue, as compare to non-transferred IL-10-/- mice. Overall, our data demonstrate that in vivo, IL-10 produced by M-MDSCs during KP35 infection is essential to establish an anti-inflammatory environment that allows host survival, despite a delayed bacterial clearance.

Phagocytosis is impaired from activation markers overexpression on monocytes from systemic inflammation patients

Luis Angel Flores-Mejía[1,2], Eduardo Antonio Ferat Osorio[2], Ismael Mancilla-Herrera[3], Arturo Cérbulo-Vázquez[4], Constantino López-Macías[2], Armando Isibasi[2], Lourdes Andrea Arriaga-Pizano[2]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Instituto Nacional de Perinatología, Mexico City, Mexico
[4]Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
anjl_72@hotmail.com; eduardoferat@me.com; mahi_25803@yahoo.com.mx; cerbulo@hotmail.com; constantino@sminmunologia.mx; isibasi@prodigy.net.mx; landapi@hotmail.com

Keywords: Cytokines, Inflammation, Phagocytosis, Sepsis, immunophenotype

SIRS-Sepsis-Septic Shock refers to a complex syndrome that represents an actual health problem in the intensive care units around the world. It has been described that some clinical signs and symptoms in Sepsis/SIRS are promoted by physiopathology changes like cytokines production, that some works classify in profiles pro-inflammatory, anti-inflammatory or mixed. Also, have been suggested an interrelationship between cytokine environment and alteration in others systems like endothelial, complement, coagulation systems, as well as leucocytes. Both overexpression of so-called activation markers (TREM-1, CD16 and CD64 on monocytes) and diminished phagocytosis and cell signaling have being reported in both patients with sepsis and animal models. However, until know, no evidence exists if the "activated immunophenotype" is related with increased functional capabilities. Our aim was to prove this relation at the single cell level.

A total of 44 patients with Systemic Inflammation (31 septic and 13 SIRS patients) were evaluated. Serum cytokines were determined by immunoassays, and immunophenotype and phagocytic activity were evaluated at single cell level using flow cytometry. As previously reported, both pro- and anti-inflammatory cytokines are increased in serum from both SIRS and sepsis, compared with sera from healthy individuals ($p = 0.0004$). As well, leukocytosis from SIRS/sepsis patients depends on neutrophilia (Healthy = 516.3 +/-139.8 cells/uL; SIRS = 1472.4 +/- 1095.0 cells/uL and Sepsis = 1236.6 +/- 423.6 cells/uL). Monocytes shown overexpression of CCR7, CD69, CD107a and CD16 ($p < 0.05$). However, phagocytic capacity showed no differences among phagocytes from SIRS/sepsis patient and healthy volunteers ($p = 0.0553$). A disable immune response

clearly occurred despite leucocyte overexpression of “activation markers”, so a more profound analysis should be made before assuming activated immunophenotype means increased leucocyte responses.

Electrophoretic pattern and Western blot analysis of the components of the filtrate cultures and soluble cell wall fraction of Mycobacterium bovis isolates from the state of Zacatecas

Gloria Guillermina Guerrero Manriquez, Blanca Patricia Lazalde, Jesus Said Garcia, Javier Jovany Sanchez

Autonomous University of Zacatecas, Zacatecas, Mexico
gloguerrero9@gmail.com; Blancalazalde@gmail.com; saidejgh@gmail.com; jovanykill@hotmail.com

Keywords: BCG Vaccine, PPD, M. bovis, Patogenicidad, Proeomica

Bovine tuberculosis is a zoonotic infection caused by Mycobaceterium bovis, a member of the complex Mycobacterium tuberculosis, that constitutes a serious heatlh and economic problem worldwide (CDC., 2005; WHO., 2012). Both organism share around 9.5% of homology, can cause identical clinical disease in humans and are genetically very similar.and it is highly possible that most of the virulence factors of M. bovis are the same as those of the classical human tuberculosis organism, Mycobacterium tuberculosis (Collins DM., 2001). That is why most of the of the immunopathogenesis knowledge regarding bTB has been extrapolated from the findings in human tuberculosis. The RD1 locus, RD1 (Rv3871-Rv3879c), a known virulence gene cluster. common to pathogenic mycobacteria is deleted in M. bovis Bacillus Calmette Güerin (BCG)(Kearns et al., 1999; Mattow et al., 2001; Inwald et al., 2003; Gao et al., 2004), encodes proteins that are actively secreted by pathogenic mycobacteria, including M. tuberculosis/M. bovis. These proteins are promising not only as candidates for vaccination and diagnostic tests, because its immunomodulatary properties, but also in understanding mycobacterial evasion of protective immunity in susceptible individuals (human sor cattles) (Trajkosvic et al., 2004). The mycobacterial reléase of immunodominant or virulence factors is posible thanks to the presence of an elegant secretory system, named 6 kDa early secretory antigenic target (ESAT6) protein family secretion (ESX) systems (also known as type VII secretion systems that helps the pathogen to resist or evade the host immune response (Brosh et al., 2001; Brodin et al., 2004; Gröschel et al., 2016). Much of the recent research have focused on this system, and in the biological characterization of mycobacteria-specific ESX/type VII secretion systems and their secreted proteins, belonging to the Esx, PE, and PPE categories (Brodin et al., 2004: Gröschel et al., 2016). Recently, there has been major progress in understanding the biogenesis, secretion and antigenicity of ESAT-6 proteins and, at least in the case of ESAT-6

system 1, in unravelling their role in pathogenicity of the members of the complex of M. tuberculosis (Brodin et al., 2004; Champion et al., 2006; Majlessi et al., 2015; Garcia et al., 2015; Tashakori et al., 2016). Moreover, MPB70 and MPB83 are among the most studied mycobacterial antigens. encoded as precursor proteins with typical signal peptides for export through the general secretory pathway. MPB70 is a soluble secreted protein cleaved by signal peptidase I (Wiker HG., 2009). They are the major antigens highly expressed by Mycobacterium bovis and considerably less abundantly expressed by M. tuberculosis. while MPB83 is a glycosylated lipoprotein processed by signal peptidase II and located at the surface, possibly with the lipid tail coupled to the N-terminal cysteine embedded in the mycobacterial outer membrane. Their expression is controled by transcriptional regulator SigK. A mutated versión of this in BCG leads to down expression of these proteins. Both proteins are immunogenic since it elicited T-cell responses and antibody responses. These properties are been extensively explored for sensitive and specific diagnosis of bovine tuberculosis (Wiker et al., 2009) with some promising results. Moreover, in the filtrate of the early cultures of M. bovis Bacillus Calmette Güerin (BCG)(used for homology to M. lepre, M. tuberculosis)(Pessolani et al, 1989) as well as in the soluble fraction of the cell wall (Reswan et al., 2007), it has been detected activities of several enzymes such as, mucinases, lipases, proteases, RNAses, proteins that bind to fibronectin (Kannan et al., 1987). However, it is not known the role of these in the immunopathogenesis of the bTB. Due to the complex interaction host –pathogen, there are still several aspects in the mechanism of immunopathogenesis that deserve further study. The objective of the present work is to analyze in detail the components of the filtrate and soluble fraction of the cell wall of two strains of M. bovis, isolated from different regions of the Zacatecas State, using one dimensional electrophoresis (SDS-PAGE), followed by serological analysis to determine the immunoreactivity of these components in order to determine the potential role in the immunopathogenesis of bTB. To do, this, M. bovis strains were grown respectively in medium 7H10/OADC/THF, 37°C/ CO2, a different times, early (7–8 days), 15 and 21 day Thereafter, a supernatant was obtained from each strain, and filtered through 0.45 μm, and one part of this was precipitated with 50 and/or 75% of ammonium sulfate. The soluble fraction of the cell wall was obtained after the second sonication of the pellet and separated after ultracentrifugation. The different fractions were analyzed in SDS-PAGE gel 12% and transferred to nitrocellulose membrane. The reactivity of the components of the differents fractions was tested with individual negative, positive serum samples from Zacatecas cattles. We found that the profile of proteins between the M. bovis strains was slightly different in certains bands, but in general both strains showed a similar pattern with enrichments of proteins from 10, 24, 53 and/or 85 kDa. Interestingly, there is a differential serological response of these bands of proteins from the negative and/ positive serum, suggesting the potential role of these proteins in the interaction cattle-M. bovis. We are in the process to extend this study to another endemic states

from Mexico, with the purpose to develop an alternative and improved immunological diagnostic method, which can be explored at more deeply level, using also other omics technologies that can strengthened the understanding of the immunopathogensis mechanism of bTB.

REFERENCES

World Organization for Animal Health. 2012.- Página web: www.oie.int/wahid-prod/ public. php?page=disease_status_detail&disease_id=32

Centers for Disease Control and Prevention (CDC). 2005. 54: 605-608. www.cdc-gov/mmwt/preview/mmwrhtml/mm5424a4htm.

Collins DM1. Tuberculosis (Edinb). 2001. 81:97-102.

Inwald J1, Jahans K, Hewinson RG, Gordon SVTuberculosis (Edinb). 2003. 83: 387-93.

Kearns AM1, Magee JG, Gennery A, Steward M, Graham C, Seiders PR, Freeman R. Int J Tuberc Lung Dis. 1999. 3:635-8.

Mattow J1, Jungblut PR, Schaible UE, Mollenkopf HJ, Lamer S, Zimny-Arndt U, Hagens K, Müller EC, Kaufmann SH. Electrophoresis. 2001. 22: 2936-46.

Gröschel MI1,2, Sayes F1, Simeone R1, Majlessi L1, Brosch R1. Nat Rev Microbiol. 2016. 14: 677-691. doi: 10.1038/nrmicro.2016.131.

Rao PK1, Li Q. Molecules. 2009. 14: 3237-58. doi: 10.3390/molecules14093237.

Brosch R1, Pym AS, Gordon SV, Cole ST. Trends Microbiol. 2001. 9: 452-458

Majlessi L1, Prados-Rosales R, Casadevall A, Brosch. RImmunol Rev. 2015. 264:25-45. doi: 10.1111/imr.12251.

Wiker HG1. Scand J Immunol. 2009. 69:492-499. doi: 10.1111/j.1365-3083.2009.02256.x.

Gao LY1, Guo S, McLaughlin B, Morisaki H, Engel JN, Brown EJ. Mol Microbiol. 2004. 53:1677-93.

Brodin P1, Rosenkrands I, Andersen P, Cole ST, Brosch R.. Trends Microbiol. 2004. 12:500-8.

Champion PA1, Stanley SA, Champion MM, Brown EJ, Cox JS. Science. 2006. 313:1632-1636.

Trajkovic V1, Natarajan K, Sharma P. Microbes Infect. 2004. 6:513-9.

Pessolani VMC, Rumjanek DF, De Melo Marques MA, De Melo SFF, and Sarno EN. 1989. J. of Clin. Microbiol. 27: 2184-2189

Rezwan M1, Lanéelle MA, Sander P, Daffé M. Microbiol Methods. 2007. 68:32-39. Epub 2006 Jul 12.

Calder B1, Soares NC, de Kock E, Blackburn JM. Expert Rev Proteomics. 2015:21-35. doi:10.1586/14789450.2015.1007046.

Tashakkori MM1, Tebianian M2, Tabatabaei M1, Mosavari N1. Int J Mycobacteriol. 2016.. doi: 10.1016/j.ijmyco.2016.10.027.

García E1, Bianco MV1, Gravisaco MJ1, Rocha RV1, Blanco FC1, Bigi F2 Tuberculosis (Edinb). 2015. 95:186-9. doi: 10.1016/j.tube.2015.01.001. Epub 2015 Jan 9.

Contribution of CD1d to immune responses and pathogenesis in mouse models of human Respiratory Syncytial Virus and the human Metapneumovirus infections

Emma Rey Jurado[1,2], Karen Bohmwald[1,2], Nicolás MS Gálvez[1,2], Daniela Ivonne Becerra Diedrichs[1,2], Steven Anthony Porcelli[3], Leandro J Carreno[2,4], Alexis M Kalergis[1,2,5]

[1]Pontificia Universidad Católica de Chile, Santiago, Chile
[2]Millennium Institute on Immunology and Immunotherapy (MIII), Santiago, Chile
[3]Albert Einstein College of Medicine, New York City, United States
[4]Universidad de Chile, Santiago, Chile
[5]INSERM UMR Nantes, Nantes, France
erey@bio.puc.cl; nerakzita@gmail.com; nrgalvez@uc.cl; nrgalvez@uc.cl; daniela.becerra.d@gmail.com; steven.porcelli@einstein.yu.edu; leandrocarreno@uchile.cl; akalergis@bio.puc.cl

Keywords: viral infection, human metapneumovirus, natural killer T cells, pulmonary inflammation, Human respiratory syncytial virus

Human respiratory syncytial virus (hRSV) and human Metapneumovirus (hMPV) cause acute respiratory tract infections in children worldwide. Natural killer T (NKT) cells are unconventional T cell lymphocytes and their TCRs recognize glycolipids bound to the MHC-I-like CD1d molecule. Here, we evaluated the contribution of CD1d and the role of these NKT cells on both hRSV and hMPV infections. A significant decrease of neutrophils and CD103+ DCs infiltration to the lungs, and higher levels of IFN-γ were found in hRSV- but not in hMPV-infected CD1d-deficient mice as compared to hRSV-infected wild-type mice, being similar to the mock-treated mice. To better understand as to how NKT cells modulate T cell immunity during these infections, we evaluated the capacity of α-GalCer-pulsed dendritic cells (DCs) to induce NKT cell activation, when previously infected with hRSV or hMPV. Interestingly, both hRSV and hMPV inhibited DC-mediated iNKT cell activation, showing reduced production of IL-2. In conclusion, wild-type mice are more resistant to hRSV- but not to hMPV-infection, thereby CD1d-dependent NKT cells induced by hRSV may be detrimental for the outcome of the infection. In addition, impairment of NKT function has been found in both viruses as a potential mechanism of immune evasion.

Variation of major immunoglobulins in septic shock. Intensive care unit, leon cuervo rubio hospital. 2015–2017

Alexander Ariel Padron-Gonzalez[1], Maricarmen González-Costa[1], Anisleidys Martínez-Infante[2], Pablo Jose Padrón-Morera[2], Alberto Juan Dorta-Contreras[3]

[1]Instituto de Ciencias Básicas y Preclínicas "Victoria de Girón". Universidad de Ciencias Médicas La Habana. Cuba
[2]Hospital León Cuervo Rubio. Pinar del Río. Cuba
[3]Laboratorio Central de Líquido Cefalorraquídeo (LABCEL) Facultad Miguel Enríquez, Universidad de Ciencias Médicas de la Habana, Cuba
paxander@infomed.sld.cu; carmenmari@infomed.sld.cu; any.martinez.24895@ucm.pri.sld.cu; alex.padron.91@ucm.pri.sld.cu; adorta@infomed.sld.cu

Keywords: Septic shock, Etiology, Epidemiology, Major Inmunoglobulins Variation

Abstract

Introduction: Sepsis syndroms are a threat for public health. Septic Shock is a subset of sepsis in which occurs circulatory, cellular, and metabolic abnormalities associated with a greater risk of mortality.

Materials and methods: an observational, descriptive, cross-sectional study was carried out in the ITU of Leon Cuervo Hospital, from January 2015 to June 2017. Target group: 107 admitted patients with septic shock in the time of the study. Sample: 75 patients with sepsis, who applied by the inclusion criteria. The information was obtained from the cases registry and personal clinical records.

Results: Septic shock predominated in people older than 65 years without significative diferences in sex. Pneumonia in the community was the main cause of hospitalization. Gram positives germs predominated and Staphylococcus were the principal microorganisms isolated. Levels of IgM and IgA were elevated meanwhile IgG levels were normal.

Discussion: The epidemiology, etiology and microbiology were similar than other studies. The administration of intravenous inmunoglobulins is controversial and futures studies should be made.

Introduction: Sepsis is a life-threatening organ dysfunction cause by a dysregulated host response to infection that injures its own tissues. It´s incidence has risen with more than 1 000 000 hospitalizations per year. Septic shock is associated with profound circulatory, cellular, and metabolic abnormalities with a greater risk of mortality. Clinically patients have respiratory rate of 22/min or greater, altered mentation, systolic blood pressure of 100mm Hg or less serum lactate level greater than 2 mmol/L in absence of hypovolemia, persisting hypotension that require a vasopressor treatment to maintain a mean arterial pressure of 65 mm Hg or greater.

Materials and methods: An observational, descriptive, cross-sectional study was carried out in the ITU of Leon Cuervo Hospital, from January 2015 to June 2017. Target group: 107 admitted patients with septic shock in the time of the study. Sample: 75 patients with septic shock, who applied by the inclusion criteria. The information was obtained from the cases registry and personal clinical records.

Results

Table 1: Age and sex of patients with septic shock admitted. Intensive Care Unit, Leon Cuervo Rubio Hospital. 2015–2017

	Sex					
Age (years)	**Female**	**%**	**Male**	**%**	**Total**	**%**
≤24	1	1,3	2	2,7	3	4
25 a 34	3	4	3	4	6	8
35 a 44	4	5,3	3	4	7	9,3
45 a 54	3	4	5	6,7	8	10,7
55 a 64	10	13,3	9	12	19	25,3
> 65	**18**	24	**14**	18,7	**32**	**42,7**
Total	39	51,9	36	48,1	75	100

Table 2: Location of infection at admission of patients with septic shock. Intensive Care Unit, Leon Cuervo Rubio Hospital. 2015–2017

Location of the infection	**No.**	**%**
Pneumonia of the community	25	**33,3**
Septicemia	16	21,3
Genitourinary sepsis.	13	17,3

(*Continued*)

Table 2: (*Continued*)

Location of the infection	No.	%
Gastrointestinal sepsis	10	13,3
Central Nervous System Sepsis.	6	8,1
Soft tissues	5	6,7

n = 75

Font: Personal clinical records

Table 3: Gram staining in samples of septic shock patients. Intensive Care Unit, Leon Cuervo Rubio Hospital. 2015–2017

	No.	%
Gram Positives	44	58,7
Gram Negatives	31	41,3
Total	75	100,0

Font: Personal clinical records

Table 4: Microorganisms isolated in samples of septic shock patients. Intensive Care Unit, Leon Cuervo Rubio Hospital. 2015–2017

Germs	n = 75	
	No.	%
Staphylococcus	30	40
Pseudomona	15	20
Streptococcus	14	18,7
Escherichia coli	6	8
Klebsiella	5	6,7
Proteus	3	4
Enterobacter	2	2,6

Font: Personal clinical records

Table 5: Levels of major Igs. Intensive Care Unit, Leon Cuervo Rubio Hospital. 2015–2017

Values of the Major Immunoglobulins						
	Low		Normal		High	
Igs	#	%	#	%	#	%
Ig M	11	14,7	17	22,7	47	**62,6**
Ig G	31	**41.3**	25	33,3	19	25,4
Ig A	10	13,3	27	36	38	**50,7**

Discussion: Septic shock was more frequent in people over 65 years old there was no significant difference in sex. It significate more than 2% of hospitalizations in the world. Respiratory infections in the community were the main reason for admission of patients with 33.3% of cases, followed by septicemia and genitourinary sepsis in that order similary to other studies. Respiratory infections account for approximately half of all cases of sepsis with a higher risk for developing respiratory organ dysfunction.

Gram Positive Germs predominated with 58.7% of cases consistent with studies in the region. The main microorganisms isolated in the cultures were Staphylococcus, Pseudomonas and Streptococcus. In the world Gram-positive bacterial infections are the most common cause of septic shock in the last 25 years. In USA more than the 50 percent of sepsis are cases of Gram-positive germs.

The Ig M remained between high and normal levels because in the initial contacts with the agents it is the first that rises which favors the activation of the complement system retains the antigens and can neutralize germs. The levels of IgG were in normal values mostly, perhaps due to the moment in which it was quantified. This immunoglobulin vital in the second response favors opsonophagocytosis, neutralizes infectious agents, is able to activate the complement. Ig A was elevated because it is the main immunoglobulin at the mucosal level where microorganisms usually penetrate. It is able to neutralize and opsonize infectious agents.

The administration of intravenous inmunoglobulins (IIVA) in patients with sepsis is controversial. Even when many metaanalyses found that IIVA in adult patients with severe sepsis or septic shock was associated with an overall survival benefit, it´s use is not recommended by the current international guidelines for the treatment of sepsis syndromes. In spite of that inmunoglobulins can be used to boost the immune response to an infection, to regulate and control an excessive inflammatory response. Maybe the high costs of its production, limited worldwide supply and the limited evidence base are the main causes that remains its use to cases in which conventional therapy has failed. Future clinical studies should be addressed in order to show the relationship between levels of Inmunoglobulins in patient with sepsis and the relevance of its intravenous administration. This advances will cause changes in the impact of sepsis syndroms.

REFERENCES

Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016 Feb 23; 315(8): 801–810. doi: 10.1001/jama.2016.0287

Seymour CW, Rosengart MR. Septic Shock Advances in Diagnosis and Treatment. JAMA. 2015 Aug 18; 314(7): 708–717. doi: 10.1001/jama.2015.7885

Martin GS. Sepsis, severe sepsis and septic shock: changes in incidence, pathogens and outcomes. Expert Rev Anti Infect Ther. 2012 Jun; 10(6): 701–706. doi: 10.1586/eri.12.50

Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM et al. Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med. 2013 Feb;39(2):165-228.

Elfeky S, Golabi P, Otgonsuren M, Djurkovic S, Schmidt ME, Younossi ZM. The epidemiologic characteristics, temporal trends, predictors of death, and discharge disposition in patients with a diagnosis of sepsis: A cross-sectional retrospective cohort study. J Crit Care. 2017 Jun;39:48-55.

Schorr CA, Zanotti S, Dellinger RP. Severe sepsis and septic shock. Virulence. 2014 Jan 1; 5(1): 190–199. doi: 10.4161/viru.27409

Chun-Ta H, Yi-Ju T, Pi-Ru T, Chong-Jen Y, Wen-Je K. Epidemiology and Outcome of Severe Sepsis and Septic Shock in Surgical Intensive Care Units in Northern Taiwan. Medicine (Baltimore). 2015 Nov; 94(47): e2136. doi: 10.1097/MD.0000000000002136.

Berlot G, Rossini P, Turchet F. Biology of Immunoglobulins. Transl Med UniSa. 2015 Jan-Apr; 11: 24–27.

Almansa R, Tamayo E, Andaluz-Ojeda D, Nogales L, Blanco J, Eiros JM et al. The original sins of clinical trials with intravenous immunoglobulins in sepsis. Crit Care. 2015; 19(1): 90. doi: 10.1186/s13054-015-0793-0

Raithatha AH, Bryden DC. Use of intravenous immunoglobulin therapy in the treatment of septic shock, in particular severe invasive group A streptococcal disease. Indian J Crit Care Med. 2012 Jan-Mar; 16(1): 37–40. doi: 10.4103/0972-5229.94433

Soares MO, Welton NJ, Harrison DA, Peura P, Shankar-Hari M, Harvey SE et al. Intravenous immunoglobulin for severe sepsis and septic shock: clinical effectiveness, cost-effectiveness and value of a further randomised controlled trial. Crit Care. 2014; 18(6): 649. doi: 10.1186/s13054-014-0649-z

Hamano N, Nishi K, Onose A, Okamoto A, Umegaki T, Yamazaki E et al. Efficacy of single-dose intravenous immunoglobulin administration for severe sepsis and septic shock. J Intensive Care. 2013; 1(1): 4. doi: 10.1186/2052-0492-1-4

IL-23R and IL-17RA levels in gingival tissue from patients with Chronic and Aggressive Periodontitis

Alondra del Carmen Ruíz-Gutiérrez, Ruth Rodriguez Montaño, María Luisa Pita-López, Martha Graciela Fuentes-Lerma, Vianeth María del Carmen Martínez-Rodríguez, Ana Lourdes Zamora, Celia Guerrero-Velázquez

University of Guadalajara, Guadalajara, Mexico
alo_crg@hotmail.com; ruth_rodriguez21@outlook.com; mpita08@gmail.com; cdomartha@hotmail.com; vianethperio@gmail.com; anazamora@gmail.com; celiagv2001@yahoo.com.mx

Keywords: Aggressive Periodontitis, Chronic Periodontitis, IL-17RA, Gingival tissue, IL-23RA

Introduction: Periodontal disease (PD) is one of the two main oral pathologies that affect the world population including Mexico (1–2). Periodontitis belongs to group II of PD, the most common types of periodontitis are chronic (CP) and aggressive (AP) and are characterized by gingival inflammation, periodontal pocket formation, insertion lost and alveolar bone destruction (2–3). The immune process is characterized by several cytokines including the IL-23 / IL-17 axis. Regarding immunopathology, periodontopathogenic bacteria induce the secretion of proinflammatory cytokines such as IL-23 by dendritic cells. IL-23 binds to its receptor (IL-23R) in Th17 cells inducing the production of IL-17 and RANKL. On the other hand, IL-17 binds to IL-17R in fibroblasts and these secrete RANKL who activate preosteoclasts differentiating them into mature osteoclasts, which are responsible for alveolar bone resorption (4–6). IL-23 receptor (IL-23R) consists of a subunit called IL-23R bound to the beta 1 subunit of the IL-12 receptor (IL-12Rβ1)7. The IL-17 family is recognized by five receptors (A-E) of which the IL-17RA heterodimer and IL-17RC recognize IL-179. In CP patients there is an increase in IL-23 and IL-17 in samples of gingival tissue (TG), plasma (P) and gingival crevicular fluid (LCG) 10–12. Also IL-23R is increased in the P of patients with AP vs CP and healthy subjects (HS). Conversely, the IL-17 receptor (IL-17RA) is elevated in the SS vs PC and PA. However, IL-23R and IL-17RA have not been studied in TG samples from patients with PC and PA.

Material and Methods

Study subjects

The protocol was approved by the Research, Bioethics and Biosafety Committees of the University of Guadalajara. The study subjects were recruited to participate of Periodontal Clinic of the Dentistry School of University of Guadalajara. The purpose

of this study was explained to each subject before he/she agreed to participate in the study, and their informed consent was obtained according to the Declaration of Helsinki and all subjects gave their written approval before participating based on the General Health Law and the NOM-008-SSA2-1993 norm.

Study groups

Medical and dental records were obtained for all participants and were diagnosed according to the classification of the American Academy of Periodontology

Healthy subjects group (HS): n = 9 (4 males / 5 females) mean age (38 ± 10)

Chronic periodontitis group (CP): n = 9 (4 males / 5 females) mean age (47 ± 13)

Aggressive periodontitis (AP): n = 9 (3 males / 6 females) mean age (32 ± 4)

Sample collecttion

Sample collection Gingival tissue samples. Inflamed gingival tissue (only from active periodontal sites) from patients was collected by a flap operation during routine periodontal surgery using a scalpel, after which the wound was secured with sutures. Prior to surgery, all individuals underwent an initial periodontal treatment phase. Collection of gingival tissue from HS was performed before tooth extraction for orthodontic / prosthetic indications. Tissues samples were frozen at -70C until analysis.

Tissue preparation

The gingival tissue (GT) was solubilized according to the techniques described by (Gorska et al, 2003; Johnson et al, 2005) Briefly, the tissue was blotted, weighed on a microbalance, cut into small pieces (1–2 mm3) with a scalpel and then placed in a 1.5-ml microtube with a sufficient volume of phosphate-buffered saline (PBS) to ensure the following dilution: 10 mg tissue/100 ll PBS plus protease inhibitor (Sigma Chemicals, St Louis, MO, USA). Subsequently, the tissue was macerated with a polypropylene pestle and a vortexer. Next, it was centrifuged at 600 g, and the supernatant was frozen at -70C until analysis by cytokine ELISA. The procedure was carried out at 4C.

Protein assay

A standard Bradford micromethod was used to assess the protein concentration in each gingival sample (Bradford, 1976). The absorbance was read at 570 nm in a microplate spectrophotometer. Protein concentrations were calculated from a bovine serum albumin

standard curve (Sigma Chemical) and were expressed as pg/ ml. The Bradford reagent used consisted of 100 mg of Coomassie brilliant blue G-250 (Research Organics, Cleveland, OH, USA), 50 ml of 95% ethanol (Caledon Laboratories Ltd, Georgetown, ON, Canada), 100 ml of concentrated phosphoric acid (Caledon Laboratories Ltd) and 200 ml of distilled water.

Enzyme-linked immunosorbent assay (ELISA)

Aliquots from either tissue homogenates or cytokine standars were added in triplicate to the wells of microtiter plates to determine the concentration of human IL-23R and IL-17RA using Duo set ELISA kit (R&D Systems, Minneapolis, MN). The absorbance was read at 450 nm in a microplate spectrophotometer.

For the statistical analysis, the SPSS v 22.0 package for Windows was used, applying a Kruskal Whalis test for the comparison of independent means of three or more groups and significant differences were observed. Later, according to the Shapiro-Wilks test, the results showed an abnormal distribution, so a nonparametric Mann-Whitney U test was applied to know the differences between the means of SS, PC, AR and ARPC and a $P<0.05$ It was considered significant.

Results: The results are presented as the mean ± the standard error of the IL-23R and IL-17RA levels (pg/mg) of gingival tissue and are represented in graphs.

The levels of IL-23R were higher in the HS group and significant differences were observed when compared with the group of PC and AP with a $P = 0.050$ and $P = 0.001$ respectively (Figure 1a). On the other hand, IL-17RA levels were higher in the AP group and only a significant difference was observed against the PC group with a $P = 0.021$ (Figure 1b).

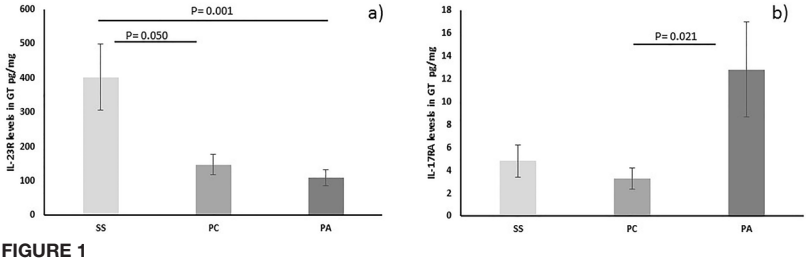


FIGURE 1

Conclusion: We observed a significant decrease in IL-23R in patients with PC and AP compared to HS. Regarding IL-17RA, it was found higher in AP compared to CP and HS in gingival tissue.

REFERENCES

1. Petersen PE, Ogawa H. Strengthening the prevention of periodontal disease: the WHO approach. J Periodontol. 2005; 76 (12): 2187-3193.
2. Craig RG, Pernat AM, Pecoits-Filho R, Levin NW, Kotanko P. Periodontal diseases and systemic infl ammation. Seminars in Dialysis. 2013; 26: 16-39.
3. Armitage GC. Development of a classifi cation system for periodontal diseases and conditions. Annals of periodontology. Ann Periodontol. 1999; 4 (1): 1-6.
4. Lindhe J, Karring T, Lang NP . Clinical periodontology and implant dentistry. 4th ed. Oxford, UK ; Malden, MA: Blackwell; 2003; XXIV: p. 1044.
5. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005; 366 (9499): 1809-1820.
6. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nature Immunology. 2005; 6 (11): 1123-1132.
7. Abdi K, Singh NJ, Spooner E, Kessler BM, Radaev S, Lantz L et al. Free IL-12p40 monomer is a polyfunctional adaptor for generating novel IL-12-like heterodimers extracellularly. J Immunol. 2014; 92 (12): 6028-6036.
8. Yang XP, Ghoreschi K, Steward-Tharp SM, RodriguezCanales J, Zhu J, Grainger JR et al. Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. Nature immunology. 2011; 12 (3): 247-254.
9. Vernal R, Dutzan N, Chaparro A, Puente J, Antonieta VM, Gamonal J. Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis. J Clin Periodontol. 2005; 32 (4): 383-389.
10. Himani GS, Prabhuji ML, Karthikeyan BV. Gingival crevicular fluid and interleukin-23 concentration in systemically healthy subjects: their relationship in periodontal health and disease. J Periodontal Res. 2014; 49 (2): 237-245.
11. Lester SR, Bain JL, Johnson RB, Serio FG. Gingival concentrations of interleukin-23 and -17 at healthy sites and at sites of clinical attachment loss. Journal of periodontology. 2007; 78 (8): 1545-1550.
12. Cifcibasi E, Koyuncuoglu C, Ciblak M, Badur S, Kasali K, Firatli E et al. Evaluation of Local and Systemic Levels of Interleukin-17, Interleukin-23, and Myeloperoxidase in Response to Periodontal Therapy in Patients with Generalized Aggressive Periodontitis. Infl ammation. 2015; 38 (5): 1959-1968.

Immunological characterization of the PE_PGRS33 protein of Mycobacterium tuberculosis

Adriana Garibay-Escobar[1], Carlos Velazquez[1], Clara Espitia[2], Esmeralda Ivonne Niño-Padilla[1], David Ortega-Tirado[1], Paola Gastélum-Aviña[1], Carmen Judith Serrano[3], Eduardo Ruiz-Bustos[1], Ramón Enrique Robles-Zepeda[1], José Antonio Enciso-Moreno[3]

[1]Universidad de Sonora, Hermosillo, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[3]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
adriana.garibay@unison.mx; velaz@guayacan.uson.mx; espitia@biomedicas.unam.mx; esmeralda.nino@unison.mx; davidortegati@correoa.uson.mx; pga88@hotmail.com; carmenyuyu2000@yahoo.com.mx; eduardo.ruiz@unison.mx; rrobles@guayacan.uson.mx; enciso2000@yahoo.com

Keywords: Latent Tuberculosis, Interferon-gamma release assay (IGRA), BCG Bacillus Calmette Guerin, PE_PGRS33 protein, T cells hybridome

Tuberculosis (TB) is a disease caused by Mycobacterium tuberculosis (Mtb), and a leading cause of mortality due to an infectious agent. In 2016, the World Health Organization reported 10.4 million new cases and 1.3 million deaths. This places TB as a worldwide health issue. The major problem is due to the lack of an effective vaccine to control the disease, since BCG is the only authorized vaccine against TB. However, its protection spectrum is highly variable in adults and adolescents (0 to 80%). For this reason, the development of a more efficient vaccine is imperative. Nowadays, the creation of proteins/peptides-based vaccines is an advantageous approach. In order to apply such approach to TB vaccinology, it is necessary to characterize the immune response towards an Mtb antigen that elicits a protective immune response against the infection.

About 5% of the Mtb genome corresponds to the PE_PGRS family of proteins. Some members of this group have proven to have immunogenic properties such as cytokine profile alteration of cells infected with the mycobacteria. One of the most studied members of the family is the PE_PGRS33 protein that is capable of triggering both cellular and humoral immune responses in mice. In addition, the PE domain of this protein is highly conserved among Mtb strains making this protein a good target for new vaccine development. Thus, the aim of our study was to characterize the immune response to PE_PGRS33. First, we produced and purified a recombinant PE_PGRS33 protein using E. coli as an expression vector. Afterwards, we isolated peripheral blood mononuclear cells (PBMC) from latent TB individuals (LTB) defined as both positive to tuberculin skin test (TST) and interferon-gamma release assay (IGRA), and controls. The PBMC were stimulated with recombinant PE_PGRS33 to determine

the production of IFN-γ from CD4+ T-cells by flow cytometry. Our results showed that 2 out of 6 LTB individuals and one non-LTB responded to PE_PGRS33 with the production of high levels of IFN-γ by CD4+T-cells and some of its synthetic peptides. CD4+T cell proliferation assesed by CFSE dilution revealed antigen-specific clonal expansion to stimuli to both protein and peptides. Based on these results, we were interested in a more detailed study about the immune response to PE_PGRS33. Therefore, PE_PGRS33-specific T-cell hybridomas were develop in order to obtain an unlimited source of cells to study in vitro immune responses by the performance of an antigen presentation assay. Through this assay, the secretion of cytokines by the T cell hybridoma was triggered. Such cytokines served to stimulate a macrophages-cell line and the secreted cytokines were characterized as well as those secreted by the PE_PGRS33 specific T-cell hybridoma. This was carried out by multiple cytokine analysis. Results showed that the PE_PGRS33 specific T-cell hybridoma yielded a pro-inflammatory cytokine profile. In contrast, macrophages showed an anti-inflammatory profile when stimulated with the cytokines from the antigen presentation assay. In summary, our results revealed that PE_PGRS33 protein can induce a cellular immune response and it may be responsible for inducing an anti-inflammatory phenotype on a cell line of macrophages.

Plasmodium berghei survives inside dendritic cells and causes malaria recrudescence in pregnancy

Oscar Murillo, Erika Machado, Carla Leticia Bandeira, Lígia Antunes Gonçalves, Sabrina Epiphanio, Claudio Romero Farias Marinho
University of São Paulo, São Paulo, Brazil
murgomez@gmail.com; e.machado@usp.br; carla.leticia@usp.br; lig.antunes.goncalves@gmail.com; sabrina.epiphanio@gmail.com; crfmarinho@gmail.com

Keywords: Dendritic Cells (DC), pregnant, P. berghei, Recrudescent, Chronic mice

Malaria during pregnancy is associated with several adverse effects on the mother, fetus, and newborn. The parasites sequester in the placenta and lead to consequent maternal anemia, intrauterine growth retardation, and decreased fetal viability [1]. Around twenty-eight million pregnant women live in endemic areas, though, only 43% receive proper treatment upon infection [2][3]. Nonetheless, most treatments are sub-curative, conducting to Plasmodium persistence despite apparently cleared in the blood, which increases the risk for recrudescence[4]. Thus, it is not fully clear how and where the parasite harbors, and which cells mediate this mechanism. Dendritic cells (DCs) are possible responsible, as have been shown to have the ability to keep the parasite alive and generate infection[5][6]. Therefore, using a murine model for recrudescence established by us [7], we searched the mechanisms behind the recrudescence triggered by pregnancy in mice previously infected by P. berghei and treated. These chronically infected female mice showed adverse effects on the placenta with high plasma levels of IL-10 upon pregnancy, despite occurring recrudescence or not. Further, we unraveled that in the non-pregnant chronic mice the P. berghei accumulate in the spleen and lungs. Additionally, FACS analysis and subsequent electronic microscopy revealed that DCs isolated from lungs and spleen expressed intracellular GFP, as we used GFP-expressing P. berghei. Furthermore, we found that depletion of DCs with clodronate abrogated the recrudescence, suggesting that DCs are responsible for keeping the parasites alive, which further recrudesce during gestation. Moreover, when infected DCs are sorted and transferred to naïve pregnant mice, and not to non-pregnant, a new blood stage infection is generated, indicating that parasites harbored in DCs are fully infectious. Remarkably, DCs from non-pregnant chronic mice showed a decrease in the activation profile, by reduced expression of CD80, CD86, and MHC-II, which are accompanied by functional loss of inducing lymphocyte proliferation even when stimulated by LPS, CpG or IRBC. Altogether, these results strongly suggest that the survival of Plasmodium berghei inside DCs can be facilitated by the impairment of the activation and functional profile of these cells in chronic infections, which evade immunological clearance by hiding in the spleen and lungs. Interestingly, the recrudescence of the hidden parasites is triggered by pregnancy, which implies the mediation of this phenomenon by pregnancy factors.

REFERENCES

1. Brabin BJ, Romagosa C, Abdelgalil S, Menéndez C, Verhoeff FH, McGready R, et al. The sick placenta-the role of malaria. Placenta. 2004;25: 359–78. doi:10.1016/j.placenta.2003.10.019
2. Shaukat AM, Gilliams E a, Kenefic LJ, Laurens MB, Dzinjalamala FK, Nyirenda OM, et al. Clinical manifestations of new versus recrudescent malaria infections following anti-malarial drug treatment. Malar J. 2012;11: 207. doi:10.1186/1475-2875-11-207
3. WHO. World malaria report 2016. 2016.
4. Laochan N, Zaloumis SG, Imwong M, Lek-Uthai U, Brockman A, Sriprawat K, et al. Intervals to Plasmodium falciparum recurrence after anti-malarial treatment in pregnancy: A longitudinal prospective cohort. Malar J. ???; 2015;14: 1–9. doi:10.1186/s12936-015-0745-9
5. Wykes MN, Kay JG, Manderson A, Liu XQ, Brown DL, Richard DJ, et al. Rodent blood-stage Plasmodium survive in dendritic cells that infect naive mice. Proc Natl Acad Sci U S A. 2011;108: 11205–10. doi:10.1073/pnas.1108579108
6. Wykes MN, Horne-Debets J. Dendritic cells: the Trojan horse of malaria? Int J Parasitol. Australian Society for Parasitology In.; 2012;42: 583–7. doi:10.1016/j.ijpara.2012.03.002
7. Marinho CRF, Neres R, Epiphanio S, Gonçalves L a, Catarino MB, Penha-Gonçalves C. Recrudescent Plasmodium berghei from pregnant mice displays enhanced binding to the placenta and induces protection in multigravida. PLoS One. 2009;4: e5630. doi:10.1371/journal.pone.0005630

Participation of MHC-II+ CD63+ exosomes derived from human monocytes in the T-lymphocytes activation by immunological synapse during Dengue virus infection

Pedro Pablo Martínez-Rojas, Verónica Monroy-Martínez, Blanca Haydé Ruiz-Ordaz

Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
pedropablo.martinezrojas@gmail.com; veromon@yahoo.com; bhro@unam.mx

Keywords: Dengue, Exosomes, T lymphocytes, Immunological Synapse, Cell activation

Introduction: Dengue fever (DF) is the most important arbovirus in tropical and subtropical regions worldwide: more than 100 million cases are reported annually. DF presents a broad clinical spectrum ranging from the self-limited form (85–90% of cases) to severe dengue (SD, 10–15%). Dengue virus (DENV) is a Flavivirus that infects mainly antigen-presenting cells (APCs): monocytes/macrophages and dendritic cells (Hasan, et al., 2016). In the SD pathogenesis, there is an increase in cellular activation, elevated levels of cytokines and extracellular vesicles (microparticles and/or exosomes), with plasma extravasation due to vascular endothelium damage (Pang, et al., 2017). Exosomes are nanovesicles derived from endosomes that serve as vehicles for different types of biomolecules, with important functions in intercellular communication processes that include the immunological synapse (IS) (van Niel, et al., 2018). Canonically, IS has been described between APCs and T-lymphocytes (TL) however, exosomes can carry in their membranes, molecules of the Major Histocompatibility Complex II (MHC-II) and tetraspanin (t) enriched microdomains (Huppa and Davis, 2003), especially with t CD63, which could function as a co-stimulatory molecule. In DENV infection, the function of MHC-II+ CD63+ exosomes derived from DENV-infected cells (exDENV) in the IS with TL has not been described and could be an important mechanism in the sustained cellular activation that induces the cytokine storm reported in the SD.

Aim: To evaluate the MHC-II+ CD63+ exDENV participation in the TL activation by IS in an in vitro model of DENV infection.

Methods: Viral amplification and titration by lytic plate assay of DENV-2 NGC strain in Macaca mulatta monkey (LLC-MK2) kidney epithelial cells (ATCC CCL-7). Kinetics

of DENV infection in human monocytes (THP-1) of peripheral blood (ATCC TIB-202) at different multiplicities of infection (MOI): E protein (viral envelope) detection by flow cytometry (FC) and evaluation of cell morphological changes by light-field optical microscopy (LF-OM). Infected-monocytes surface markers (CD11b, CD14, CD63 and MHC-II) evaluation by FC. Extracellular vesicles isolation by differential ultracentrifugation. The exDENV characterization by nanoparticle tracking analysis (NTA, concentration [particles/mL] and size [20–150 nm]), transmission electron microscopy (TEM), and MHC- II & CD63 detection by FC. Co-culture assays of MHC-II+ CD63+ exDENV with a) naïve THP-1 cells to determine their activating effect through CD11b detection by FC and TNF-α expression by RT-PCR and agarose gel electrophoresis, and b) human TL (Jurkat, clone E6-1) of peripheral blood (ATCC TIB-152) to evaluate: 1) IS by immunohistochemistry (with and without anti-TCR-α/β-1 blockade) and lymphoproliferation by MTT assay, 2) mRNA transcription factors expression (T-bet, STAT-1/-4) by RT-PCR with agarose gel electrophoresis, and 3) IL-2 and IFN-γ quantification by ELISA.

Results: The cytopathic effect during DENV amplification in LLC-MK2 cells, consisting in syncytia formation, was shown between 7 and 12 days post- infection (PI). A viral titer of 1.0 E+7 PFU/mL was obtained. In the THP-1 cells infection kinetics, the best condition achieved was MOI = 1 at 72 h PI, in which the viral E protein was detected at a mean fluorescence intensity (MFI) of 30-fold higher than Mock cells. Morphologically, Mock cells were kept in suspension with similar lymphoblast characteristics while DENV-infected cells presented heterogeneous changes such as anisocytosis, decrease in the nucleus-cytoplasm ratio and adherent phenotype. At 72 h PI, compared in terms of Mock cells MFI, CD11b was found increased 96.2-fold while CD14 and CD63 decreased 4.0 and 2.2-fold, respectively. There was no significant change in the MHC-II expression. Nanoparticles (20–150 nm) concentration of 7.3 E+10 ± 6.5 E+9 particles/mL derived from DENV-infected cells and 1.51 E+10 ± 7.1 E+9 particles/mL derived from Mock cells were obtained ($n = 6$, $P < 0.05$). TEM images analysis shown heterogeneous population of extracellular vesicles with abundant structures compatible with exosomes: defined vesicles at a 100 nm scale with a double regular bilayer; electrodense bodies were observed inside and/or associated with the exDENV membrane and not were observed in exosomes derived from Mock cells (exMock). This was confirmed by FC: more than 80% of exDENV populations were MHC-II+ CD63+. In exDENV with naïve THP-1 cells co-culture assays at 72 h post-stimulus (PS), compared in terms of Mock cells MFI, the CD11b expression in exDENV-stimulated cells was 63.0-fold higher. Morphologically, cellular changes were observed from 24 h PS, showing the maximum transformation level at 72 h PS: these changes included mainly adherent

phenotype formation with cytoplasmic projections. TNF-α expression was observed at 24 h PS. Preliminary immunohistochemistry assays have shown interaction between exDENV and TL. The rest of the experimental strategy is in development.

Conclusion: So far, these data show that MHC-II+ CD63+ exDENV are obtained in high concentrations as a product of activation of DENV-infected cells and their interaction with naïve cells induce activation with TNF-α expression. exDENV participation in the IS with TL is promising.

REFERENCES

Hasan, S., Jamdar, SF., Alalowi, M., and Al Ageel Al Beaiji, SM. (2016) Dengue virus: A global human threat: Review of literature. J. Int. Soc. Prev. Community. Dent. 6(1): 1-6. doi: 10.4103/2231-0762.175416

Pang, X., Zhang, R., and Cheng, G. (2017) Progress towards understanding the pathogenesis of dengue hemorrhagic fever. Virol. Sin. 32(1): 16-22. doi: 10.1007/s12250-016-3855-9

van Niel, G., D'Angelo, G., and Raposo, G. (2018) Shedding light on the cell biology of extracellular vesicles. Nat. Rev. Mol. Cell. Biol. doi: 10.1038/nrm.2017.125

Huppa, J., and Davis, M. (2003) T-cell antigen recognition and the immunological synapse. Nat. Rev. Immunol. 3: 973-983. doi: 10.1038/nri1245

Primary infections with hRSV promotes mycobacterial colonization and lung pathology in mice

Gisela Canedo-Marroquín, Pablo F Céspedes, Susan Bueno Ramirez, Alexis M Kalergis

Pontificia Universidad Católica de Chile, Santiago, Chile
Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom
gecanedo@uc.cl; pfcespedes@outlook.com; susan.bueno@gmail.com; akalergis@bio.puc.cl

Keywords: Mycobacterium bovis, respiratory disease, HRSV, coinfection., mycobcaterial colonization

Worldwide, the human respiratory syncytial virus (hRSV) is the leading cause of severe lower respiratory tract infection in infants. Because recurrent epidemics based on reinfection occur in children and adults hRSV has gained interest as a potential primary pathogen favouring secondary opportunistic infections. Infections with hRSV are associated with poor development of cellular immune responses, including the generation of T-dependent neutralizing antibodies. Furthermore, potential deleterious modulation of antiviral immunity driven by cytokines secreted by lung epithelial cells may have unexpected negative effects on the immune response to subsequent infections. Here, we evaluated whether hRSV blunts the host immune response to subsequent pathogens using intranasal instillation of Mycobacterium bovis as a model for secondary infections. Eleven days-post infection we evaluated immune cells populations by flow cytometry and the expression of key genes involved in immune surveillance by RT-PCR. Also, we determine loads of viable bacilli by direct culture of lung homogenates. Our data suggest that previous infection with hRSV significantly increases the susceptibility to bacterial respiratory pathology characterized by increased infiltration of neutrophils and high counts of myeloid cells with an inflammatory macrophage profile, which in last term correlates with increased colony-forming units (CFUs) of M. bovis. Our data suggest that hRSV impairs the local pulmonary immune response favoring bacilli colonization and the development of mycobacterial pneumonia in infected individuals.

Humoral and cellular mother immune response against Toxoplasma gondii and their relation to vertical transmission and to clinical outcome in their congenitally infected newborns

Fernando Gómez-Chávez[1,2], Yevel Flores-García[1], Diana Miriam Barrios-Bautista[1], Christian Alejandro Bonilla-Ríos[1], Valeria Gómez-Toscano[1], Emmanuel Arce-Estrada[1], Irma Cañedo-Solares[1], Hector Luna-Pastén[1], Dolores Correa[1]

[1]*Instituto Nacional de Pediatria, Mexico City, Mexico*
[2]*Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico City, Mexico*
fergocha@gmail.com; yvlnfg@yahoo.com.mx; bdianami@gmail.com; christianbonri@yahoo.com.mx; valeria_172884@yahoo.com; emmanuel_ares@hotmail.com; casoli2001@gmail.com; hec_vene@hotmail.com; mariadol@yahoo.com

Keywords: Pregnancy, Toxoplasma gondii, cytokine production, congenital toxoplasmosis, Humoral and cellular immune response

Toxoplasmosis is a parasitic zoonosis widely distributed, caused by the ingestion of contaminated water/food with Toxopasma gondii. If a pregnant woman is infected with this parasite, it can be transmitted to the fetus inducing ocular, neurologic, or systemic damage with a variable range of severity (1). The profile and intensity of mother´s immune response has been suggested as an important factor determining the rate of parasite transmission and clinical severity in the congenitally infected newborn (2, 3). The aim of this work was to establish a possible relation between the maternal immune response and vertical transmission or infection dissemination/severity in the infected newborn. Thirty-six pregnant women were recruited from the Instituto Nacional de Perinatología, Mexico City, Mexico. The subjects ranged in age from 18 to 39 years. All patients selected in this study had no diagnosis for other chronic or acute infection or auto-immune disease according to the clinical profile records, any of them received specific drug treatment at the sample collection time but as soon as the diagnosis was confirmed, they were all treated up to delivery with spiramycin or pyrimethamine. They were diagnosed as positive for toxoplasmosis based on IgG and IgM antibodies and/or parasite DNA by ELISA/ WB and B1 gene qPCR (3). We also searched for IgG1, IgG2, IgG3, IgG4 and IgA specific antibodies by ELISA and we isolated peripheral blood mononuclear cells for measuring specific cell proliferation induced by T. gondii, by CFSE and lymphocyte subpopulations staining with the antibodies anti: CD3/APC-Cy7, CD4/APC, CD8/PERCP-Cy5, CD19/PE-Cy7 (BD Bio-sciences, Mountain View, CA, USA). Cytokines production from these cells was measured using the human inflammatory cytokine multiplex bead array kit (Cat. no. 551811)

and human Th1/Th2 (Cat. no. 551809) for detection of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IFNγ, TNF-α, and TGFβ (Cat. no. 560429). Cell proliferation and cytokine production were analyzed in a FACSAria II BD using FlowJo v7 software (Tree Star Inc., Ashland, OR, USA) and the FCAP Array software from BD. Subsequently, we followed their pregnancies until delivery and blood samples were collected from their newborns to verify congenital infection by serology and qPCR, allowing us classify mothers into non-transmitter and transmitter women. In parallel, newborns were clinically evaluated to establish congenital toxoplasmosis clinical features (localization and severity outcome).

We found that levels of IgG2-4 subclasses, IgA anti-Toxoplasma antibodies and lymphocyte proliferation, specially CD3+ cells, were higher in mothers who transmitted the infection in comparison to those who did not. Furthermore, IgG1, IG3, IgA and IL-8 were found increased in transmitter mothers with newborns in whom disseminated/severe toxoplasmosis developed, rather than in those who delivered infected newborns with localized-neurological/low clinical outcome. Our results are consistent with the paradoxical previous reported data in humans and mice models of congenital toxoplasmosis, which suggest that the immune profile that protects mothers from a disseminated or severe disease, promotes transmission and damage in the fetus.

REFERENCES

Abou-Bacar A, Pfaff AW, Georges S, Letscher-Bru V, Filisetti D, Villard O, et al. Role of NK Cells and Gamma Interferon in Transplacental Passage of Toxoplasma gondii in a Mouse Model of Primary Infection. Infect Immun (2004) 72, 1397-1401. doi:10.1128/IAI.72.3.1397-1401.2004

Cañedo-Solares I, De La Luz Galván-Ramírez M, Luna-Pastén H, Pérez LRR, Ortiz-Alegría LB, Rico-Torres CP, et al. Congenital toxoplasmosis: specific IgG subclasses in mother/newborn pairs. Pediatr Infect Dis (2008) J 27, 469-474. doi:10.1097/INF.0b013e31816591df

Dubey JP, Jones JL. Toxoplasma gondii infection in humans and animals in the United States (2008). Int J Parasitol 38, 1257-1278. dog:10.1016/j.ijpara.2008.03.007

Aminoguanidine Prevents Actinomycetoma development by Nocardia brasiliensis in Mice

Mario Cesar Salinas-Carmona, Humberto R garza-Esquivel, Ossian Longoria-Lozano, Juan A López-Ulloa, Anna Velia Vazquez-Marmolejo

Facultad de Medicina, Universidad Autonoma de Nuevo León, Monterrey, Mexico
mario.salinas@uanl.mx; hrge_123@hotmail.com; ossianlongoria@gmail.com; jlopezulloa@gmail.com; velvazmar63@yahoo.com.mx

Keywords: Cytokines, iNOS, d, Nocardia brasiliensis, Actinomycetoma, Aminoguanidine

Introduction: Aminoguanidine (AG) is a selective chemical inhibitor of inducible nitrate oxide synthase enzyme (iNOS), this compound was extensively used to investigate the role of nitric oxide (NO) in vascular damage in diabetes. It was also used to demonstrate that activated macrophages can destroy Mycobacterium tuberculosis with the production of oxygen and nitrogen free radical intermediates (RNI and NOI). Using aminoguanidine it was demonstrated the importance of these free radicals in killing different intracellular bacteria. Inducible NOS is produced by macrophages under LPS, TNF-α and IFN-γ stimulation, leading to a high local NO concentration; high level of the NO, contributes to tissue damage in chronic inflammatory infections.

Aim: In the present work, we studied the effect of NO in actinomycetoma development in BALB/c mice infected with Nocardia brasiliensis by blocking iNOS with aminoguanidine.

Materials and Methods: BALB/c mice received aminoguanidine hemisulfate (2.5% w/v) in drinking water, ad libitum. Animals treated with this iNOS inhibitor showed no actinomycetoma development, reinfection with the pathogen only produced transient inflammation but fail to establish the actinomycetoma lesion. Antibody production and cytokine secretion were determined by ELISA; T cell proliferation was quantitated by tritiated thymidine incorporation and flow cytometry to identify cytokine producing cells. Spleen size of aminoguanidine treated and infected animals was compared to non-treated animals.

Results: The infected foot pad of treated rodents, showed no inflammation, compared with infected but non AG treated animals. These dramatic reduction of tissue inflammation, equals to other experimental mice that developed typical full-blown actinomycetoma and were then, treated with aminoguanidine in water. Spleen size of infected non treated animals is 3 to 4 times higher than volume of AG treated animals. Spleen cells recovery from AG treated group was also decreased and showed a great effect in CD4 lymphocytes, AG treatment had no effect in anti Nocardia brasiliensis antibodies titer.

Conclusion: Aminoguanidine treatment prevents actinomycetoma development and heals full-blown lesion and has no effect in antibody production nor T lymphocyte proliferation.

IL-1α promotes liver inflammation and necrosis during blood-stage Plasmodium chabaudi malaria

Maria Menezes, Flavia Sarmento Vieira, Érika Machado Salles, Eduardo Amaral, Alexandra Cassado, Jose M Alvarez, Jose Carlos Alves-Filho, Maria Regina D'Império Lima

University of São Paulo, São Paulo, Brazil
menezesnmaria@gmail.com; flavinhafsv@gmail.com; erika.salles@gmail.com; eduardo.amaral@nih.gov; alecassado@hotmaill.com; jmamosig@icb.usp.br; jcafilho@usp.br; relima@usp.br

Keywords: Inflammation, Malaria, Plasmodium chabaudi, liver damage, IL-1alpha

Background & Aims: The malaria-induced liver damage may lead to the release of damage-associated molecular patterns (DAMPs). Once in the extracellular environment, DAMPs can contribute to inflammation. IL-1α and IL-1β are important cytokines from the IL-1 family that can act as alarm signals. The present study aims to evaluate the role of these cytokines in the liver pathology caused by malaria.

Methods: C57BL/6 and IL1A-/- male mice were infected with 1x106 Plasmodium chabaudi-infected erythrocytes. The liver was removed and submitted to histopathological, gene expression, cytokine quantification and cellular analysis.

Results: P. chabaudi infection led to substantial increase in IL-1α mRNA and protein in the liver and this effect was NLRP3 inflammasome-independent. In contrast, hepatic IL-1β levels were not affected by the infection. Leukocytes are a major source of IL-1α in the liver of infected C57BL/6 mice. Although IL-1α deficiency had no effect in the peak of parasitemia, it resulted in reduced weight loss and lower hypothermia. Furthermore, infected IL1A-/- mice showed lower liver necrosis, pro-inflammatory cytokine production and cellular infiltration compared to infected C57BL/6 mice.

Conclusion: This study shows that leukocytes are a main source of IL-1α in the liver during acute P. chabaudi malaria. This cytokine promotes liver necrosis and inflammation in response to infection, as well as exacerbates the weight loss and hypothermia.

Association of Single-nucleotide polymorphisms in immune-related genes with development of Dengue Hemorrhagic fever in a Mexican population

Angélica Vargas-Castillo[1], Karina Ruiz-Tovar[2], Alejandro Escobar-Gutiérrez[2], Héctor Vivanco-Cid[3], Salvador Fonseca-Coronado[4]

[1]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico
[2]Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), México, Mexico
[3]Universidad Veracruzana, Xalapa, Mexico
[4]Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autonóma de México, Cuautitlán Izcalli, Mexico
bere_aj@hotmail.com; karinaruiztovar@yahoo.com.mx; aescobargutierrez@yahoo.com; hvivanco@hotmail.com; fonsecacoronado@yahoo.com

Keywords: Dengue, Mexican population, Single nucleotide polymorphisms (SNPs), TNF alpha, Immune related genes

Background: Dengue viruses cause a wide range of clinical manifestations. Mechanisms responsible for dengue-related disease outcome are not fully understood but immune-related molecules play an important role in triggering severe dengue. Single nucleotide polymorphisms (SNPs) in immune-related genes have been associated with risk for development of dengue, or for protection from acquiring it, depending on ethnicity.

Aim of the study: To genotype seven SNPs located in genes of the immune response in order to identify their association with severe forms of dengue in patients from an endemic region of Mexico.

Methods: One hundred and thirty eight patients with dengue fever (DF) and 31 Dengue Hemorrhagic Fever (DHF) patients as well as 304 healthy donors were genotyped by qPCR using TaqMan probes for the SNPs: rs1800629 (TNF-α gene), rs4804803 (DC-SIGN gene), rs2780831 (JAK1 gene), rs1801274 (FcγRIIa gene), rs231775 (CTLA-4 gene), rs12979860 and rs8099917 (IL-28B gene).

Results: The rs1800629 A-Allele in TNF-alpha gene was associated with DHF (OR=3.072, CI=1.023–9.223, p=0.0374) while rs4804803, rs2780831, rs1801274, rs231775, rs12979860 and rs8099917 SNPs showed no association in the cohort studied.

SNP	Group	fA	Numberof Alleles	MAF%	Allele test	
					OR (95% CI)	p-value
DC-SIG	HD(n=304)	G	0/304	0		
rs4804803	DF(n=138)		0/304	0	-	-
	DHF(n=31)		0/304	0	-	-
TNF-α.	HD(n=304)	A	579/29	4.8		
rs1800629	DF(n=l38)		266/10	3.6	0.750(0.360-1.563)	0.0398
	DHF(n=31)		55/7	11.29	**3.385(1.235-9-284)**	**0.0212***
JAKI	HD(n=304)	A	276/332	54.6		
rs2780831	DF(n=138)		118/158	57.2	1.113(0.8354-1.483)	0.5111
	DHF(n=31)		26/36	58.1	1.084(0.5918-1.807)	1.000
FcγRIIa	HD(n=304)	G	287/321	52.8		
rs1801274	DF(n=138)		135/141	51.1	0.933(0.7024-1.241)	0.6631
	DHF(n=31)		29/33	53.2	1.090(0.6273-1-892)	0.7803
CTLA-4	HD(n=304)	G	297/311	51.2		
rs231775	DF(n=l38)		128/148	53.6	1.104(0.8303-1.469)	0.5138
	DHF(n=31)		26/36	58.1	1.198(0.6859-2.091)	0.5739
IL-28B 0	HD(n=304)	T	306/302	49.7		
rs1297986	DF(n=l38)		133/143	51.8	1.089(0.8195-1.448)	0.5623
	DHF(n=31)		25/37	59.7	1.377(0.7864-2.409)	0.3242
IL-28B	HD(n=304)	G	332/276	45.4		
rs8099917	DF(n=138)		152/124	44.9	0.9813(0.737-1.306)	0.9419
	DHF(n=31)		31/21	50	1.226(0.7061-2.128)	0.4838

*Statistical significance. SNP, Single Nucleotide Polymorphism; HD, Healthy Donors; DF, Dengue Fever; DHF, Dengue Hemorrhagic Fever; MA, minor allele; MAF, Minor allele frequency; N, number of individuals; OR, Odds ratio; IC, Confidence Interval.

Conclusions: These results demonstrates that allelic variations in TNF-alpha could play a role in the development of DHF but the lack of association between all remaining SNPs and DHF suggests that genetic background might directly modify the role of these immune-related molecules that lead to the milder illness often observed in Mexican population.

Bacillus Calmette-Guerin infection induces granuloma-like structures in humanized BLT-Lung mice

Claire Johnson, Laura Rank, Rachel Cleary, Miriam Braunstein, Angela Wahl, J.Victor Garcia

University of North Carolina at Chapel Hill, Chapel Hill, United States
claire3@email.unc.edu; rank@email.unc.edu; clearyr@email.unc.edu; miriam_braunstein@med.unc.edu; angela_wahl@med.unc.edu; victor_garcia@med.unc.edu

Keywords: Tuberculosis, mouse model, human immunodeficiency virus, humanized mice, Bacillus Calmette–Guerin (BCG)

Mycobacterium tuberculosis (TB) is one of the ten most prevalent causes of death in the world and is a significant world health concern, particularly in conjunction with human immunodeficiency virus (HIV). Approximately 36 million people worldwide are living with HIV. Of those, about one-third have latent TB. TB is the most common cause of death among those with HIV, and TB treatment is less effective in individuals with HIV. In Latin America, about 13% of deaths among HIV-infected individuals is TB-related.

The development and testing of effective vaccines and therapeutics for TB would be enhanced by an in vivo model that reflects the human condition. Mice are commonly used as a TB animal model, but they are limited in that the granulomas formed do not show all the hallmarks of human granulomas. Conversely, non-human primate, rabbit, and guinea pig models of TB can form more human-like granulomas but are limiting in cost and availability of immunological reagents. Importantly, none of these models are susceptible to HIV. As such, an improved model is necessary for the study of HIV/TB co-infection. Our goal is to develop a novel animal model that will more closely mimic human HIV/TB co-infection.

In humans, the lung is the primary site of TB infection. Here, we implanted human lung tissue subcutaneously into the back of immune deficient (NSG) mice to create lung-only mice (LoM) with human lung organoids. To evaluate the susceptibility of human lung organoids to mycobacterium, we inoculated Bacillus Calmette-Guerin (BCG), an attenuated Mycobacterium bovis strain used as the live vaccine strain for TB, directly into human lung organoids of LoM. At necropsy (4–6 weeks post-exposure), we measured BCG levels in human lung organoids and spread to other organs by plating organ homogenates for viable bacteria and acid-fast staining of tissue sections.

In addition, we determined the presence of granulomas by histology. To assess the effect of a systemic human immune system on BCG persistence and spread in vivo, we implanted autologous human lung tissue into bone marrow-liver-thymus (BLT) humanized mice (BLT-L mice). BLT mice are systemically reconstituted with human immune cells and have been extensively used to study HIV infection in vivo. BLT-L mice possess human lung organoids and autologous systemically distributed innate and adaptive human immune cells. Following inoculation of BCG into human lung organoids of BLT-L mice, we measured BCG levels in human lung organoids, systemic spread, and granuloma formation at necropsy (4–6 weeks post-exposure).

Our results revealed that in both the LoM and BLT-L mice, BCG persisted and disseminated to distant organs (liver, spleen, and mouse lung) after inoculation into human lung organoids. Importantly, in the presence of systemic human immune cells (BLT-L mice), we observed granuloma-like structures in multiple organs. In the future, we will confirm the susceptibility of human lung organoids to TB infection and use LoM and BLT-L mice to evaluate the role of the immune system in the control of TB infection in vivo. In addition, using BLT-L mice, we will evaluate the effect of HIV infection on TB replication and spread as well as granuloma formation in vivo. In summary, our data validates a novel in vivo model for studying human mycobacterial infection which could be used in the future for the design, evaluation, and optimization of TB prevention strategies, therapeutics, and vaccines.

Identification of Giardia lamblia molecules that contribute to mast cell activation and cytokine secretion

Samira Muñoz Cruz[1], Argelia Gómez-García[1], Felix Matadamas-Martínez[1], Patricia Meza-Cervantez[1], Juan Antonio Alvarado-Torres[2], Lilian Yépez-Mulia[1]

[1]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
mcsamira@yahoo.com.mx; mundechocolate@yahoo.com.mx; felixmatadamas@yahoo.com.mx; patriciamezc@gmail.com; aquarius_at89@hotmail.com; lilianyepez@yahoo.com

Keywords: Giardia lamblia, mast cell, mast cell activation, innate immune response, proinflamatory citokines

Mast cells play a central role in the early clearance of the intestinal protozoan parasite Giardia lamblia. The importance of mast cell in the control of Giardia infections has been clearly elucidated; however, the understanding of how Giardia activates mast cell response is incomplete. Particularly concerning to how mast cell recognizes the parasite and the identification of the parasite molecules involved in mast cell activation.

In a previous study, we reported that G. lamblia live trophozoites or trophozoite-derived total soluble extract induced mast cell direct activation (IgE-independent) and release of IL-6 and TNF-alpha, two key cytokines required for control of G. lamblia infection.

To identify the Giardia molecules involved in mast cell activation, trophozoite-derived total soluble proteins separated into three fractions (F1-F3) were evaluated for its ability to activate mast cell in vitro.

Here, we demonstrated that F2 fraction induced mast cell activation and enhanced mast cell release of IL-6 and TNF-alpha, in a greater extent than F1 or F3 fraction. F2 electrophoretic analysis showed at least 20 protein bands of moderate to high intensity, five of them were unique to this fraction, while seven high intensity bands were shared with F1 and F3 fractions. Proteins in unique and high intensity bands from F2 were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Several G. lamblia immunodominant proteins, as well as metabolic enzymes, were identified among the major proteins present in the F2 fraction.

Finally, to determine Giardia specific proteins that induce mast cells activation, we tested mast cells response to recombinant enolase and arginine deiminase (ADI), two major immunodominant proteins secreted in vivo and in vitro during G. lamblia infection. Recombinant ADI significantly increased mast cell release of TNF-alpha and IL-6.

In conclusion, in the present study we demonstrated that Giardia F2 fraction and Giardia-ADI induced mast cells activation and proinflammatory cytokine production, although the specific mechanism for the activation of mast cells by these Giardia molecules has yet to be elucidated.

Virulence and immunopathology induced by Mycobacterium tuberculosis Beijing and Beijing like strains isolated from Colombian Patients

Maria Irene Cerezo Cortes[1], Dulce Adriana Mata[2], Estela Isabel Bini[2], Martha Isabel Murcia Aranguren[1], Brenda Marquina-Castillo[2], Rogelio Hernandez Pando[2]

[1]National University of Colombia at Bogotá, Bogotá, Colombia
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
irenecerezo@gmail.com; dulmat@yahoo.com.mx; estelabini@yahoo.com.ar; mimurciaa@ynal.edu.co; brenda.marquina@gmail.com; rhdezpando@hotmail.com

Keywords: Colombia, Tuberculosis, Virulence, Beijing genotype, Immune reponse, Citoquines, Mycobacterium tubrculosis

Introduction: Mycobacterium tuberculosis Beijing family is an emergent pathogen widely dispersed across the world. In Colombia a variant of this genotype, the Beijing Like SIT 190 strains are the third most frequently isolated genotype after LAM and Harlem, becoming an important public health concern due to the high virulence and drug resistance characteristics of this aggressive bacteria.

Beijing genotype was originally described in 1995 by Van Soolingen et al., however this genotype has evolutionated since 6000 years ago and with the availability of genomic typing methods and DNA sequencing this genotype is classified in six ancestral clonal complexes and one sublineage of recent evolution. In Colombia the circulation of Beijing genotype has been reported since 1997 and are characterized by the absence of the 1 to 34 and 40 spacers using spoligotyping as typing method and are identified as Beijing Like strains.

Methods and Materials: The clinical isolate Beijing like SIT 190 strain 323 was isolated from a 15 years old female patient from the Colombian pacific coast, who died of tuberculosis (TB). This strain was confirmed as a multidrug resistant (MDR) isolate and was previously sequenced, point mutations were detected in the RDRs regions. A classical Beijing strain 391 was used to compare the in vivo virulence and induced immune response. Additionally, a fully drug susceptible LAM9 clinical isolate (the most prevalent in Colombia) was used as a clinical control.

For this study, the progressive pulmonary TB model in BALB/c mice was used; groups of 50 male animals per group were anaesthetized with sevoflurane (100 uL per mice) and infected by intratracheal inoculation with 250.000 bacteria/100uL of each strain. At day 1, 3, 7, 14, 21, 28 and 60 (with surviving mice) after infection groups of six mice were euthanized by exanguination under anesthesia with pentobarbital. Left lungs lobes and spleens were removed for CFU processing and histopathology.

Results: Mice infected with the Beijing Like 323 strain showed significant higher virulence than the classical Beijing strain 391; while the LAM 9 strain exhibited almost the same virulence than the Beijing like 323 strain. Higher virulence of Beijing like strain was characterized by earlier and higher mortality, higher pulmonary bacillary loads with extensive tissue damage (pneumonia). In contrast, classical Beijing 391 strain induced slower disease progression, allowing animals live up to day 60, however at this day the pulmonary histology showed necrotic areas, but with lower bacillary burdens than the animals infected with Beijing Like or LAM9 strains.

Conclusions: The high virulence of Beijing genotype was confirmed in this study, although the Beijing like strains are causing TB almost exclusively in Colombia, it is and emergent pathogen that is an important issue for the TB control in this country that can eventually disseminate to other countries producing significant public health problems. This strain also constitute an interesting model to study diverse immunopathology aspects of pulmonary TB.

Partial immunological characterization of the 5G8 protein of Giardia lamblia

Thania Maria Garzon-Lizarraga, Raul Ignacio Rascon-Samaniego, Victor Hugo Dominguez-Corrales, Maria Lucila Rascon-Duran, Jael Teresa De Jesús Quintero-Vargas, Maria Lourdes Valencia-Peña, Adriana Garibay Escobar, Carlos Velazquez

Universidad de Sonora, Hermosillo, Mexico
thaniagarzon18@gmail.com; rascons.raul@gmail.com; vhdomin92@gmail.com; lucila.rascon@unison.mx; jael.quintero@guayacan.uson.mx; lourdes.valpe@gmail.com; adriana.garibay@unison.mx; velaz@guayacan.uson.mx

Keywords: Giardia lamblia, Humoral Immune Response, B-Cell epitopes, Immunogenic antigen, Variant Surface Protein

Giardiasis is one of the most common gastrointestinal worldwide diseases. The causative agent of this disease is the intestinal parasite Giardia lamblia. Immunogenic antigens of the parasite have been identified, however, few of those molecules have been studied to know their protective role. Recently, our group identified an immunogenic protein of G. lamblia, a Variable-specific Surface Protein denominated 5G8, which induces a strong adaptive immune response in a mouse model. The main aim of this study was to characterize the humoral immune response induced by the 5G8 protein of G. lamblia in a mouse model. Herein, we generated monoclonal and polyclonal antibodies specific to the 5G8 protein from G. lamblia infected and immunized mice, respectively. Antibody recognition against several G. lamblia strains expressing different levels of the 5G8 antigen (GS/M-83-H7-5G8 (80%), GS/M-83-H7 (<5%), and WB-Clone C6 strains (0%)) was evaluated by flow cytometry and Western blotting analysis. G. lamblia Infected and immunized mice with the strains GS/M-83-H7-5G8 and GS/M-83-H7 recognized mainly the protein 5G8. The monoclonal and polyclonal antibodies against 5G8 antigen induced a strong in vitro agglutination of G. lamblia trophozoites. We identified potential linear B-cell epitopes of the 5G8 protein by using bioinformatics tools. Immunological characterization of the 5G8 protein of G. lamblia will contribute to a better understanding of the host-parasite interactions, and the possible development of a prophylactic measure against this infection.

B cells sustein Trypanosoma cruzi specific CD8+ T cell response via IL-17

Facundo Fiocca Vernengo, Cristian Gabriel Beccaria, Laura Almada, Cintia Araujo Furlan, Carolina Lucia Montes, Eva V Acosta Rodriguez, Adriana Gruppi

Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI CONICET), Cordoba, Argentina ffiocca@fcq.unc.edu.ar; cbeccaria@fcq.unc.edu.ar; lauraalmadal@gmail.com; caraujo@fcq.unc.edu.ar; cl_montes@hotmail.com; eacosta@fcq.unc.edu.ar; agruppi@fcq.unc.edu.ar

Keywords: B cell, CD8 T cell, IL-17, Anti-CD20, T. cruzi

Introduction and Objetives: Anti-CD20 therapy has been widely used to treat different diseases such as autoimmune disorders or B cell malignancies. Although B lymphocytes are the only cells capable of producing antibodies, antigen presentation and cytokine production has been attributed to these cells. Many reports highlight the role of B cells in promoting cellular immunity; however, if B cells affect CD8 responses remain uncharacterized.

To test this cell interaction we used the experimental model of T. cruzi infection, where the CD8+T cell response play a central role in the defense against this parasite.

Methods: For this, 8 days before intraperitoneal (ip) infection with 5000 trypomastigotes of T.cruzi Tulahuén strain, C57BL/6 mice were ip injected with anti-CD20 (BcD mice), to deplete B cells, or with control isotype. At 20 days post infection (dpi), tissue parasitic DNA quantification was assessed by real time PCR and T.cruzi-specific CD8+T cell response was measured by FACS using tetramers loaded with the parasite immunodominant peptide Tskb20. For in vivo cytotoxicity assay unpulsed, Tksb20, Tskb18 or PA8 pulsed spleen cells were stained with different dyes, transferred to treated or control infected animals and studied in the spleen, liver and blood cells 4 hours later by FACS. For in vitro cytokine production assay total splenocytes from either control or depleted animals were cultured for 5 hours with Tskb20, PMA-Ionomicin or left unstimulated Phenotype and function of CD8+ T cells were also analyzed by FACS. In some experiments B cell depletion was assessed 12 days after T cruzi infection. Murine recombinant IL-17 was used to treat BcD mice.

Results: Infected BcD mice exhibited higher parasitism in the spleen, liver and heart than controls. Further, infected BcD mice had a significant lower frequency and number of total and Tskb20+CD8+T cells in blood, spleen and liver ($p<0.01$, $p=0,002$ and $p=0,02$ respectively). Interestingly BcD mice presented lower frequencies of short-lived (CD44+KLRG1+CD107-) ($p=0,03$) and memory (CD62L-CD44+) ($p=0,02$) effector cells, and a significant higher frequency of naïve (CD62L+CD44-) CD8+T cells, than infected controls. Total and T.cruzi-specific CD8+T cells from infected BcD mice exhibited a lesser extent of activation but higher levels of inhibitory receptors such as LAG-3, TIGIT and PD-1. Additionally, CD8+T cells from BcD mice express lower levels of the proliferation marker Ki67 and where more apoptotic than the control counterparts. Moreover, Bcl6 expression, which can regulate CD8 T cell proliferation, was decreased in CD8+T cells from BcD mice.

When functionality was studied, CD8+T cells from infected BcD mice presented reduced cytotoxicity ($p=0,03$), degranulation, IFN**γ** and TNF production ($p<0,001$). Accordingly to the IFN**γ** production, T-bet expression was also diminished in CD8+T cells from BcD mice ($p=0,001$).

When infected mice with a settled down specific-CD8+T cell response (12dpi) were depleted from B cells, interestingly, they exhibited the same characteristics than those depleted before infection. Considering that B cells produce IL-17 during T. cruzi infection, we measured IL-17 production in the spleen cells of BcD mice and found a significative reduction of these cells.

Finally, injection of recombinant IL-17 rescued the frequency, phenotype and function of CD8+T cells generated in B cell absence.

Conclution: The results indicate that B cells are key for T. cruzi specific CD8+T cell maintenance and function, but are not necessary for their induction. Considering B cells produce IL-17 in T. cruzi infection probably its function on CD8+ T cells depends on IL-17.

Dehydroepiandrosterone supplementation modulates inflammatory response in lungs of obese and insulin resistant mice

Hernández-Bustamente I[1], Santander-Plantamura YA[2], Bini EI[1], Torres MP[2], Rodríguez-Basso A[2], Hocht C[2], Marquina-Castillo B[1], Mata-Espinosa D[1], Barrios-Payán J[1], Hernández-Pando R[1], Carranza MA[2]

[1]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México DF, México
[2]Cátedras de Farmacología y Anatomía e Histología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina
el-geco@gmail.com; Santander.yan@gmail.com; estelabini@yahoo.com.ar; torressuarez@gmail.com; angelesrbasso@gmail.com; christianhocht@hotmail.com; b_marquina@yahoo.com; dulmat@yahoo.com.mx; qcjbp77@yahoo.com.mx; rhdezpando@hotmail.com; carranza.ma@gmail.com

Keywords: Corticosterone, Diabetes Mellitus, Type 2, Inflammation Mediators, Lung Diseases, Metabolic Syndrome X

Metabolic syndrome is a cluster of risk factors, including visceral obesity, glucose intolerance, insulin resistance, hypertension, reduction of high-density lipoprotein (HDL) cholesterol and low grade chronic inflammation. Chronic pulmonary inflammation is therapeutically treated with glucocorticoids, the most widely used anti-inflammatory agents.

Although the adrenal cortex is the principal source of circulating GC, in adipocytes the enzyme 11 beta-hydroxysteroid dehydrogenase (11 b-HSD1) amplifies local GC production. It has been demonstrated that metabolic syndrome favors GC production enhancing 11 b-HSD1 expression/ activity in extra-adrenal tissues.

Adaptative humoral and cellular immunosuppression has been restored with glucocorticoid receptor antagonist and modulators of glucocorticoids like the hormone dehydroepiandrosterone (DHEA).

We investigated the effect of DHEA supplementation in an experimental model of metabolic syndrome induced by western type diet (high fat plus fructose) evaluating metabolic and inflammatory parameters.

C57-BL6/J BJ mice were randomly divided into 2 groups and feeded with control chow or high fat diet (45% of calories as fat) plus fructose (10% v/v in tap water)

during 7 mouths. Then both groups were divided in 4 groups: control diet (c), control diet supplemented with DHEA (0.05% w/w) equivalent to 60 mg/kg/24 hs (D), high fat –fructose diet (FF) and FF supplemented with DHEA (0.05% w/w) (FFD) during another 3 months. At the end of the treatment, insulin resistance was evaluated by weight registration and adiposity index, oral glucose tolerant test (OGTT), plasma insulin, triglycerides, total cholesterol and LDL cholesterol. Lung inflammation was evaluated by protein expression of TNF-a and IL-6 and 11 beta HSD1 in lung tissues by Western blot technique.

FF diet induced overweigh, glucose intolerance, insulin deficiency, and high levels of glycaemia, triglycerides and LDL-cholesterol related to C diet. Although DHEA treatment did not induced weight loss, adiposity index was restored to control levels in FFD mice. Plasma insulin was significantly increased and glucose was diminished in FFD mice respect FF group suggesting that DHEA was able to reverse the insulin resistance induced by FF diet.

In lung, FF diet significantly increased TNF-a and IL-6 respect to C diet, and DHEA induced an even greather increased expression of inflammatory cytokines in FFD mice respect to FF mice. The expression of 11 beta HSD1 enzyme was increased in lung of FF mice respect to C lungs meanwhile DHEA treatment diminish the expression in lungs of FFD mice related to FF mice.

These results suggest that in type 2 diabetes the most susceptibility to infections, including tuberculosis, could be due to that the high expression of inflammatory cytokines is contrasted by the over production of corticosterone by 11 beta-HSD1 with the finality to inhibit the pulmonary inflammation and avoid the tissular damage. DHEA treatment increased inflammatory cytokines at the same time that reduced the corticosterone activation by 11 beta HSD1 suggesting that could be useful as adjuvant therapy in infectious diseases.

In vitro antigenicity of Plasmodium vivax RON2 and Pv12 T-cell epitopes selected by their HLA-DRB1 binding profile

Carolina López[1,2], Yoelis Yepes-Pérez[2,3], Diana Diaz-Arevalo[2], Manuel Alfonso Patarroyo[1,2]

[1]Universidad del Rosario, Bogotá, Colombia
[2]Fundación Instituto de Inmunología de Colombia, Bogotá, Colombia
[3]Universidad Nacional de Colombia, Bogotá, Colombia
carolopez2880@gmail.com; yoelisyepes@gmail.com; dianadiazarevalo@gmail.com; mapatarr.fidic@gmail.com

Keywords: Plasmodium vivax, synthetic peptide, epitope, Antigenicity, Hla-DRB1 typing, Pv12, PvRON2

The development of an effective antimalarial vaccine has been delayed by the parasite's biological characteristics, including its genetic variability, the antigenic diversity, as well as the host's class II major histocompatibility complex huge diversity and haplotype frequency among the different populations.

Plasmodium protein identification has led to advancing in the selection of potential vaccine candidates and, despite the number of candidates available for Plasmodium vivax is lower due to the lack of a continuous in vitro culture, several orthologue proteins to those P. falciparum candidates have been described in the last few years.

The antigenicity of P. vivax PvRON2 and Pv12 T-cell epitopes was analyzed in the present study. Peptides were selected in silico by their predicted binding to different HLA-DRβ1* alleles, using the NetMHCIIpan-3.0 software. Eleven peptides displaying high predicted binding scores were further assayed in vitro for their binding to purified HLA-DRB1*0401, HLA-DRB1*0701, HLA-DRB1*1101 and HLA-DRB1*1302 molecules.

Peptides displaying the best in vitro binding were further tested in antigenicity assays with Peripheral Blood Mononuclear Cells (PBMCs) samples collected from individuals typed for their HLA-DRβ1* genotype, living in P. vivax malaria-endemic regions from Colombia. A peptide displaying the best affinity was selected for each allele, as well as one universal epitope from each protein, displaying the best overall binding for the four class II alleles studied (peptide 39153 from PvRON2 and peptide 39114 from Pv12) (Tables 1 and 2).

PBMCs lymphoproliferation of samples processed from the volunteers belonging to the different groups were carried out (Figure 1). CD4+ T-cell proliferation was assessed by flow cytometry, testing different stimuli, and cytokines present in culture supernatants were quantified with a commercial kit. Antigenicity assay results led to select four T-cell epitopes as potential vaccine candidates (39047 and 39154 for PvRON2, as well as 39113 and 39117 for Pv12).

Table 1. T-epitopes selected *in silico* and *in vitro Pv*RON2 binding.

	Peptide Code	Sequence	HLA-DRB1* alelle	NetMHCII pan 3.0 (%Rank)	Binding percentage*	IC50 ratio
***Pv*RON2**	39153	MKLLQHIPA NLLENI	DRB1*0401	0.5	61.20	2.93
			DRB1*0701	0.1	85.41	0.32
			DRB1*1101	6.5	72.65	10.92
			DRB1*1302	0.1	91.89	1.43
	39154	LKFIVRGNN LKFLNN	DRB1*0401	11.0	22.24	ND
			DRB1*0701	4.5	43.69	ND
			DRB1*1101	3.0	24.91	ND
			DRB1*1302	0.2	90.07	0.88
	39047	RGPVNYHFS NYMNLD	DRB1*0401	16.0	59.80	2.80
			DRB1*0701	10.0	90.41	0.26
			DRB1*1101	37.0	0.00	ND
			DRB1*1302	13.0	37.79	ND

Data from this study; ND means that a peptide had less than 50% binding so that its IC50 value was not evaluated. Rank values were considered weak binders rank ≤10 and strong binders ≤2. IC50 values were calculated for each control peptide with each DRB1 allele, the controls HA-DRB1*0401 IC50=19.44 mM; TT-DRB1*0701 IC50=23.37 mM; HA-DRB1*1101 IC50=4.77 mM; TT-DRB1*1302 IC50=7.46 mM. IC50 ratio less than 10 were considered good binders.

Table 2. T-epitopes selected *in silico* and *Pv*12 *in vitro* binding.

	Peptide Code	Sequence	HLA-DRB1* alelle	NetMHCII pan 3.0 (%Rank)	Binding percentage*	IC50 ratio
Pv12	39113	EECFLQGFN LSGKKE	DRB1*0401	3	95.81	0.38
			DRB1*0701	24	8.89	ND
			DRB1*1101	27	62.88	8.08
			DRB1*1302	55	9.28	ND
	39114	YNKIFYAR VPQRIYQ	DRB1*0401	8.5	87.38	2.22
			DRB1*0701	0.7	93.87	1.84
			DRB1*1101	0.9	82.09	1.74
			DRB1*1302	8.5	69.37	9.83
	39117	LGIIEVLI PSLPKKI	DRB1*0401	14	33.08	ND
			DRB1*0701	8	98.56	0.49
			DRB1*1101	9.5	51.13	28.04
			DRB1*1302	11	95.42	0.89

Data from this study; ND means that a peptide had less than 50% binding so that its IC50 value was not evaluated. Rank values were considered weak binders rank ≤10 and strong binders ≤2. IC50 values were calculated for each control peptide with each DRB1 allele, the controls HA-DRB1*0401 IC50=19.44 mM; TT-DRB1*0701 IC50=23.37 mM; HA-DRB1*1101 IC50=4.77 mM; TT-DRB1*1302 IC50=7.46 mM. IC50 ratio less than 10 were considered good binders.

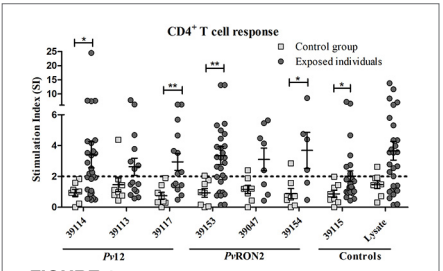


FIGURE 1

Effect of human milk sulfated Glycosaminoglycans on HIV-1 infection of human CD4+ cells in vitro

Dante Ruiz[1], Saida Briones-Peña[2], Damaris P Romero-Rodríguez[3], Geiser Cuellar-Rivera[2], Monica Viveros-Rogel[4]

[1]Universidad Autónoma Metropolitana, Mexico City, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
[3]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
[4]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
DTA_2390@hotmail.com; sbrionesp@cinvestav.mx; damaquim@gmail.com; gcuellar@cinvestav.mx; moviveros@gmail.com

Keywords: Chemokines, Glycosaminoglycans, HIV-1, human milk, Chemokines Receptors, Viral entry

Background: HIV-1 entry into its target cell CD4+ is a process that involves multiple steps. The initial interaction of HIV-1 with the cell occurs mainly by non-specific binding to lectins, heparan sulfate proteoglycans (HSPG) that are glycosaminoglycan chain-linked proteins (GAGs), glycolipids, or by ligand-receptor interactions of the virion with cell surface proteins (1–3). Sulfated glycoconjugates as glycolipids and GAGs present on the surface of colonic, vaginal epithelial and neuroglial cells bind to HIV GP120 envelope glycoprotein, suggesting that these glycoconjugates may have a role in HIV entry and infection. GAGs are polyanionic polysaccharides that are found ubiquitously in cell membranes, in the extracellular matrix and some fluids such as human milk. Chondroitin Sulfate (CS) and sulfated Heparan Sulfate/Heparin are the main species of GAGs found in human milk (4). Previous in vitro studies show that human milk GAGs (HMGAGs) inhibit HIV gp120 binding to its host cell CD4 receptor and have a potent inhibitory effect on CD4+ cells infection by HIV-1 laboratory strains and primary isolates (5–9). It has also been reported that human milk decreases the expression of chemokine receptor and HIV co-receptor: CCR5 in human PBMCs (10), so that HM-GAGs could also have an effect on CD4+ cells that contribute to inhibit HIV-1 infection by downregulation CCR5 expression and preventing HIV-1 entry. The aim of this study was to evaluate if HMGAGs alter the expression of HIV-1 main receptor: CD4 and CCR5 and CXCR4 co-receptors and the production of their ligands: MIP-1α, MIP-1β, RANTES and SDF-1 chemokines, in HIV-1 infected CD4+ cells and to define if there is a correlation with the inhibition of HIV-1 infection in vitro.

Methods: HM-GAGs were extracted from 2L of pooled human milk, purified by ion exchange chromatography and characterized by Electrospray Ionization

time-of-flight HPLC-mass spectrometry. HIV-1 IIIB (lympho-tropic virus) strain was used for inhibition assays using MT2 human T cell line. Inhibition assays were performed by a 2 hr preincubation of serial dilution (1–35μg) of HM-GAGs and shark cartilage CS (for comparison with non-human GAGs), with virus supernatant before infecting cells by 2 hr at 37°C. After 5 days, HIV-1 P24 antigen and chemokines were quantified by ELISA in culture supernatant. Significant inhibition of viral infectivity was defined as ≥80 reduction in P24 concentration with respect to the control. To determine whether exposure to HM-GAGs alters cell surface levels of HIV-1 receptors and/or co-receptors, MT2 cells from the inhibition assays were analyzed by FACS for expression of CD4, CXCR4 and CCR5. We tested 6 groups of samples in inhibition assays: cells controls, glycoconjugates controls (cells in the presence of CS and HM-GAGs), infection controls (infected cells without glycoconjugates) and samples to test (infected cells in the presence of HM-GAGs and CS). Data were expressed as the mean ±SD of triplicate wells for each condition per experiment and are representative of 3 experiments. Analysis of data was performed by one way Anova test. Statistically significant difference in HM-GAGs-treated cells as compared with controls were $P \leq 0.05$.

Results: HM-GAGs significantly inhibit MT2 infection by HIV-1 IIIB (90–100%) with respect to the maximum inhibition observed with CS (61%). No significant difference was observed in MIP-1α, MIP-1β, RANTES and SDF-1 chemokines levels neither CCR5 and CXCR4 expression between groups. There was a significant decrease in CD4 expression in infection controls and infected MT2 in the presence of low concentrations of CS and HM-GAGs (1–10μg) where the inhibition was <80%. We did note that CD4 downregulation is reversed only at highest HM-GAGs dosis (15–35μg) when inhibition was ≥90%.

Discussion: Our results suggest that strong inhibitory effect of HM-GAGs on HIV-1 infection is mainly due to its interaction with viral envelope because no effect was observed on the expression of chemokine receptors: CCR5 and CXCR4 neither MIP-1α, MIP-1β, RANTES and SDF-1 levels in MT2 cells. CD4 downregulation due to HIV-1 was reversed when infection was significantly inhibited by the highest concentrations of HM-GAGs and this effect was not observed in cells treated with CS. The strong inhibitory activity against HIV-1 infection in vitro by HM-GAGs support the possibility that they may act as soluble receptor homologues, preventing HIV-1 binding with CD4+ target cells.

Conclusion: Due to the potent inhibitory effect that HM-GAGs have shown in HIV-1 infection in vitro of CD4+ cells, they could be proposed as potential HIV-1 entry inhibitors and they may qualify as efficient microbicides agents for the virus.

REFERENCES

1. Barré-Sinoussi, F., et al. (2013). Past, present and future: 30 years of HIV research. Nature Reviews Microbiology 11(12): 877-883.
2. Wilen CB., Tilton JC., Doms RW. (2012). Molecular Mechanisms of HIV Entry. Adv Exp Med Biol, 726:223-242.
3. Fanales-Belasio, E., et al. (2010). HIV virology and pathogenetic mechanisms of infection: a brief overview. Annali dell'Istituto superiore di sanita 46(1): 5-14.
4. Coppa GV., Gabrielli O., Bertino E. et al (2013). Human milk Glycosaminoglycans: the state of the art and future perspectives. Ital J Pediatr 39:2-5.
5. Newburg, D.S. (1999). Human milk glycoconjugates that inhibit pathogens. Current medicinal chemistry 6(2): 117-128.
6. Newburg DS., Linhardt RJ., Ampofo SA. and Yolken RH. (1995). Human milk glycosaminoglycans inhbit HIV glycoprotein gp120 binding to its host cell CD4 receptor. J Nutr, 125: 419-424.
7. Viveros-Rogel M., Soto-Ramirez LE., Chaturvedi P., Newburg DS., Ruiz-Palacios GM. (2004). Inhibition of HIV-1 Infection in vitro by human milk sulfated glycolipids and glycosaminoglycans. Adv Exp Med and Biol., 554:481-487.
8. Acosta-Blanco I., Ortega-Francisco S., Dionisio-Vicuña M., Hernandez-Flores M., Fuentes-Romero L., Newburg DS., Soto-Ramirez LE., Ruiz-Palacios GM and Viveros-Rogel M. (2011). Anti-HIV Effect in vitro of Human Milk Glycoconjugates. Glycobiology Journal, 21(11): 1475 (Abstract 76).
9. Rosas-Alquicira G., Acosta-Blanco I., Ortega-Francisco S., Fuentes-Romero L., Newburg DS., Ruiz-Palacios GM., Mao Y., Zaia J. and Viveros-Rogel M. (2012). Human milk Glycosaminoglycans inhibit HIV-1 primary isolate in vitro infection. Glycobiology Journal, 22(11): 1632 (Abstract 331).
10. Lyimo MA., Howell Al., Balandya E., Eszterhas SK and Connor RI. (2009). Innate factors in human breast milk inhibit cell-free HIV-1 but not cell-associated HIV-1 infection of CD4+ cells. J Acquir Immune Defic Syndr, 51:117-124.

Higher frequencies of activated naive CD4+CD32a+ T cells in newborn and infants from HIV+ mothers

Ismael Mancilla-Herrera[1], Rodrigo T Camacho-Pacheco[1,2], Maricruz González-Aguilar[1,2], Alma Herrera-Salazar[1], Moisés León-Juárez[1], Carlos Domínguez-Vanegas[1,2], Diana Soriano-Becerril[1], Noemi Plazola-Camacho[1], Ricardo Figueroa-Damían[1]

[1]Instituto Nacional de Perinatología, Mexico City, Mexico
[2]Faculty of Chemistry, National Autonomous University of Mexico, Mexico City, Mexico
mahi_25803@yahoo.com.mx; rodrigo.tonalli@gmail.com; mary_glez1407@hotmail.com; ahs_marh@yahoo.com.mx; moisesleoninper@gmail.com; charlydominguezvanegas@gmail.com; dianasoriano@hotmail.com; noemiplazola76@yahoo.com; rfd6102@yahoo.com.mx

Keywords: memory T cells, Vertical transmission, Vertical infection, HIV reservoirs, pediatric HIV-1

Risk of vertical transmission in utero of Human Immunodeficiency Virus (HIV) has decreased with the implementation of detection programs, control of delivery mode and maternal antiretroviral (ART) medication (Luzuriaga and Mofenson, 2016). In addition, immediate ART treatments has been use to prevent the establishment of possible viral reservoirs in newborn and infants. Even though these practices, there are evidences that viral load can appear after ART cessation showing the presence of reservoirs that have not been detected (Deng and Siliciano, 2014;Luzuriaga et al., 2015;Luzuriaga and Mofenson, 2016). HIV-1 infects preferentially memory CD4+ T cells, and recently has been described a competent proviruses reservoir in these blood cells that express CD32a in HIV+ adult population (Schnittman et al., 1990;Descours et al., 2017). The objective of this study was determinate if CD4+CD32a+ T cells were present in blood of newborns and infants from ART-treated HIV+ mothers and deepen on the phenotype of these cells. We used a multi-parametric flow cytometry panel to quantify the frequencies of CD4+CD32a+ T cells, determinate their naive (CD45RA+) or memory (CD45RO+) phenotype and their expression of CD69 as activation marker, in peripheral blood samples of 13 newborns and 48 infants (2 to 19 months) from HIV+ mothers. As control, we included samples of infants from HIV-negative mothers (n=4, 2 to 19 months old) and, as references, samples of healthy (n=14) and HIV+ ART-treated (n=13) adults. Compared with healthy, the percentage of CD4+CD32a+ T cells is higher in HIV+ ART-treated adults (6.8+/-4.3 vs 2.8+/-0.9). In the same way, percentages of CD4+CD32a+ T cells in newborns (5.0+/-2.5) and infants (7.1+/-4.5) from HIV+ mothers were higher than control infants (4.2+/-1.0). Furthermore, we observed that frequencies of CD4+CD32a+ T cells in infants from HIV+ mothers increased with the age, and the most of them have naive phenotype (74.4+/-16.8) with

greater expression of CD69 in comparison with their CD32a- counterparts (177.1+/-86.2 vs 80.7+/-20.8). In summary, higher frequencies of T helper CD32a+, with naive phenotype and higher CD69 expression, are presented in peripheral blood of newborn and infants from HIV+ mothers, but future studies should be performed to evaluate if these cells could be latently HIV-infected reservoirs.

REFERENCES

Deng, K., and Siliciano, R.F. (2014). HIV: Early treatment may not be early enough. Nature 512, 35-36.

Descours, B., Petitjean, G., Lopez-Zaragoza, J.L., Bruel, T., Raffel, R., Psomas, C., Reynes, J., Lacabaratz, C., Levy, Y., Schwartz, O., Lelievre, J.D., and Benkirane, M. (2017). CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. Nature 543, 564-567.

Luzuriaga, K., Gay, H., Ziemniak, C., Sanborn, K.B., Somasundaran, M., Rainwater-Lovett, K., Mellors, J.W., Rosenbloom, D., and Persaud, D. (2015). Viremic relapse after HIV-1 remission in a perinatally infected child. N Engl J Med 372, 786-788.

Luzuriaga, K., and Mofenson, L.M. (2016). Challenges in the Elimination of Pediatric HIV-1 Infection. N Engl J Med 374, 761-770.

Schnittman, S.M., Lane, H.C., Greenhouse, J., Justement, J.S., Baseler, M., and Fauci, A.S. (1990). Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. Proc Natl Acad Sci U S A 87, 6058-6062.

Expression of taenia solium cysticercus antigenic proteins for cysticercosis immunodiagnosis

Sandra Gomez-Fuentes[1], F Sánchez[2], A Ochoa-Leyva[1], Laura Adalid Adalid-Peralta[1]

[1]*Instituto Nacional de Neurología y Neurocirugía (INNN), Mexico City, Mexico*
[2]*National Autonomous University of Mexico, Cuernavaca, Mexico*
sammxmx@hotmail.com; fili@ibt.unam.mx; adriana8a@gmail.com; adalid.laura@yahoo.com

Keywords: Recombinant Proteins, Taenia solium, bioinformatics, western blot, Secretome, Immunodiagnostic

Introduction: Neurocysticercosis (NC) is caused by the establishment of Taenia solium larvae in the central nervous system (CNS), severely affecting the health of the host. Currently, NC diagnosis is based on radiological and serological tests, however, due to its low predictive capacity, serology is only a subsidiary factor to establish a diagnosis. This work is aimed to evaluate the diagnostic value of T. solium cysticercal secretion products.

Objective: To recombinantly express T. solium excretion-secretion proteins (ESP) to develop a high-sensitivity and high-specificity neurocysticercosis immunodiagnostic method.

Results: ESP were identified by two complementary strategies: by proteomics, using two-dimension gels and Western blot, and by bioinformatics, using the algorithms, Antigenic propensity, Bepipred, Cbtop, and SVMTrip to identify antigenic regions. Additionally, the AAR (Antigenic Abundance Region) ratio, defined as the number of amino acids between antigenic regions in a protein, was used. This allowed us to identify five ESP with potential immunogenic interest. All five ESP were cloned in seven Escherichia coli strains (C41, C41 pRARE, C41 pGRO, C43, C43 pRARE, C43 pRIL, and Rossetta II) to find an appropriate expression strain for an optimal production of the recombinant protein of interest.

Long-term persistence of Zika virus infection in the eye

Nathaniel Schramm, Rae Ann Spagnuolo, Adam Cockrell, Orrin Thayer, Christopher Nixon, Ralph Baric, Martina Kovarova, J. Victor Garcia

University of North Carolina at Chapel Hill, Chapel Hill, United States
njschramm32@gmail.com; raeann_spagnuolo@med.unc.edu; adam_cockrell@med.unc.edu; orrin_thayer@med.unc.edu; christopher_nixon@med.unc.edu; rbaric@email.unc.edu; martina_kovarova@med.unc.edu; victor_garcia@med.unc.edu

Keywords: mouse models, ZIKV, persistent ZIKV infection, CD4+ T cell depletion, CD8+ T cell depletion

Zika virus (ZIKV) is a mosquito-transmitted small enveloped positive-stranded RNA virus from the Flavivirus genus that has emerged as a human pathogen with epidemic potential. Recent outbreaks in the Americas in 2015–2016 revealed that ZIKV infections, especially Asian-lineage strains, can become chronic and cause more severe clinical consequences than previously reported, including Guillain-Barre syndrome in adults and microcephaly, congenital malformation, and ocular damage in fetuses and newborn infants. Contributing to the rapid spread of the virus, and unlike most other flaviviruses, ZIKV has the potential for significant horizontal transmission due to shedding in bodily fluids. Critically, recent ZIKV outbreaks have resulted in persistent infections detected more than six months after the peripheral clearance of the virus. Developments in the transmissibility and pathogenicity of ZIKV highlight the need for appropriate animal models to study the course of infection and to test targeted therapies.

Predominant ZIKV mouse models, such as αIFNAR1 antibody treated Rag1−/− (AIR) and Ifnar1-/- C57BL/6 mice, are severely immunocompromised and result in rapid weight loss and mortality following ZIKV exposure. While important to the study of acute infection, these mouse strains fail to model chronic and persistent infection. We sought to fill this gap by characterizing an immune competent mouse model which would be more suitable for investigating long-term persistent infection once ZIKV-RNA has been cleared from the periphery.

A group of 10 Balb/c mice were intravenously exposed to 5.0 x 10^5 FFU ZIKV H/PF/2013. Two days after exposure, all animals were viremic with an average viral load of 4.9 x 10^3 ± 3.2 x 10^3 RNA copies/mL plasma. By ten days post exposure, all exposed animals had cleared ZIKV-RNA in the periphery to undetectable levels (limit of detection: 833 copies/mL). Plasma was monitored weekly for the first month after

infection, and no viral rebound was detected. The mice were tested again 201 and 283 days post exposure to confirm long-term suppression of viremia. To investigate the possible role of immune cell-mediated control of viral replication, we began depletion of CD4+ and CD8+ T cells for a period of 42 days. We confirmed depletion of CD4 and CD8 T cells by flow cytometry during weekly peripheral blood collection. No viral rebound was detected in the periphery during this time, supporting the predominance of the innate immune system in suppressing ZIKV in immune competent mice.

Finally, 329 days post ZIKV exposure and after 42 days of CD4+ and CD8+ T cell depletion, necropsy was performed and tissues of high ZIKV replication were collected for analysis of localized viral persistence in the eight animals remaining in the study. We collected and processed for analysis spleen, brain, epididymis, testes, female reproductive tract, and the eye to assess persistence of cell-associated ZIKV-RNA. The virus was undetectable in all tissues tested except the eyes. ZIKV-RNA was detected in 2/8 eyes analyzed. Our results demonstrate that despite efficient control and clearance in the periphery, ZIKV can persist in the body for almost a year post exposure.

Fetal exposure to ZIKV results in severely detrimental developmental defects. These include a number of ocular malformations including atrophy, retinopathy and glaucoma. Our results demonstrating ZIKV in the eye many months after it has been cleared from the periphery show the importance of immune privileged sites in the body where ZIKV can persist.

Evidence that normal to high glucose supplementation modifies the inhibitory effect of cortisol on the in vitro anti-TB response

Rocío Fernández[1,2], Ariana Díaz[1,2], Bettina Bongiovanni[2], Diego Bértola[3], Walter Gardeñez[3], María Victoria Pent[3], María Luisa Bay[2], Oscar Bottasso[2], Luciano D'Attilio[1,2]

[1]Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Argentina
[2]Instituto de Inmunología Clínica y Experimental de Rosario (CONICET), Rosario, Argentina
[3]Hospital Provincial del Centenario, Rosario, Argentina
rocio_26fernandez@hotmail.com; ariana_d@hotmail.com.ar; bettinabongiovanni@gmail.com; diegoabertola@gmail.com; walgarde@hotmail.com; mavipent@hotmail.com; bay.mahuad@yahoo.com.ar; oscarbottasso@gmail.com; lucianodada@yahoo.com.ar

Keywords: Glucose, Tuberculosis, Pulmonary, cortisol, Immune-endocrine interaction, Type 2 diabetes mellitus.

Tuberculosis (TB) is a major health problem worldwide and the leading cause of death by an infectious agent, Mycobacterium tuberculosis (Mtb). In 2016, WHO reported 10.4 million new TB cases [1], 15% of them attributed to co-morbidity TB plus type 2 diabetes mellitus (T2DM)[2]. In fact, T2DM may increase more than 3 times the possibility of developing active TB, which otherwise develop in 5–10% of Mtb-infected individuals (one third of the world population). Our previous results in patients with pulmonary TB and T2DM (TB+T2DM) showed a more pronounced adverse immune-endocrine profile than those with TB alone; for instance, much higher levels of IFN-γ, IL-6 and cortisol[3].

Infectious processes are a source of host stress leading to the activation of the hypothalamic-pituitary-adrenal axis (HPA) which increases cortisol production. Besides its metabolic functions, cortisol acts as an extrinsic regulator of the immune response (IR) inhibiting, at high concentrations, the proinflammatory response as well as the specific cellular IR against Mtb[4]. As such, we analysed whether high doses of cortisol (mirroring a stress situation) modified the Mtb-induced response of blood mononuclear cells (PBMC) of patients with TB+T2DM when compared to the ones yielded by TB cases, patients with T2DM (T2DM) and healthy subjects (HCo), in presence of physiological and supraphysiological glucose concentrations, or not.

Subjects (age range 18–70 years), which were all HIV negative and recruited after signing the written informed consent, were distributed as follows: TB+T2DM (n=10), TB (n=36), T2DM (n=13) and HCo (n=40). Each participant was evaluated for the

routine laboratory assays along with the circulating levels of steroid hormones, and the Mtb-driven lymphoproliferative capacity of the PBMC [Mtb strain H37rv killed by γ radiation (Mtbi)]. While the three patient groups had a significant increase in the number of leukocytes ($p<0.0001$), values in T2DM patients remained within the normal range. Both groups of TB patients showed increased and decreased relative amounts of neutrophils and lymphocytes, respectively, in comparison to HCo and T2DM (i.e., neutrophils: TB+T2DM vs. T2DM $p<0.005$; lymphocytes: TB+T2DM vs T2DM $p<0.002$). Glycemia and HbA1c concentrations were increased in the two groups of patients with T2DM, even more in those with TB+T2DM that showed the highest values (i.e. HbA1c $p<0.0001$ vs. TB). About adrenal steroids, TB and TB+T2DM patients displayed increased cortisol concentrations in presence of decreased amounts of DHEA-S, respect HCo and T2DM ($p<0.04$, both comparisons). Regarding lymphoproliferation, T2DM patients presented the highest response (T2DM vs. HCo $p<0.01$) with TB patients showing the lowest levels (TB vs. HCo, or TB+T2DM $p<0.01$). In a parallel study 1x106 PBMCs/ml from a smaller number of age- and sex-matched cases of the four study groups (TB+T2DM, n=5; TB, n=4; T2DM, n=6; and HCo, n=6) were cultured in RPMI 1640+ 5% SAB/well and stimulated with Mtbi, in presence of glucose [D-Glucose –Glc- 5 mM a physiological dose, 10, 20 or 40 mM supraphysiological doses), or not; with or without cortisol (GC 1 μM). After 24h of culture, supernatants were obtained and IL-1β levels were quantified using commercial ELISA kits. Culture supernatants from the four study groups displayed increased IL-1β amounts upon Mtbi stimulation, no matter the Glc dose; with GC treatment reducing such cytokine levels in all cases (i.e., TB+T2DM-Glc 5mM: Mtbi vs. Mtbi+GC, $p<0.003$). In analysing IL-1β relative changes respect the Mtbi-stimulated cultures (100% response), the GC-mediated inhibitory effect seen in the 4 experimental groups remained unmodified by the several Glc doses. Although TB+T2DM cells presented the lowest relative amounts of IL-1β, independently of Glc doses respect the remaining groups (i.e., Glc 20Mm: TB+T2DM vs. HCo $p<0.01$, vs. T2DM $p<0.04$, and vs. TB $p<0.03$).

Since macrophages (Mø) are target cells of Mtb infection, studies were then expanded by analysing the effect of these treatment combinations on Mø derived from the cell line THP1 (n=6, in each case). In line with the above results, culture supernatants from GC exposed Mø, showed lower IL-1β levels regardless of the Glc doses (Glc 20Mm: Mtbi+GC vs Mbti $p<0.008$). Interestingly, IL-1β levels correlated negatively with Glc doses in Mtbi-stimulated cultures added with GC or not (i.e., Mtbi+GC: $r=-0.69$, $p=0.002$; n=18).

Present results can be taken to imply that in situations accompanied by augmented GC concentrations like stress, the inhibitory effect of this compound on the immunoinflammatory response appears more pronounced in patients with the

TB+T2DM comorbidity. In turn, a hyperglycaemic status may also contribute to the GC anti-inflammatory in the early steps of the anti-TB immune response.

REFERENCES

1. World Health Organization WH. Global tuberculosis report 2016. 2016:201. doi:ISBN 978 92 4 156539 4.
2. Lonnroth K, Roglic G, Harries AD. Improving tuberculosis prevention and care through addressing the global diabetes epidemic: From evidence to policy and practice. Lancet Diabetes Endocrinol. 2014;2(9):730-739. doi:10.1016/S2213-8587(14)70109-3.
3. Fernández R, Díaz A, D'Attilio L, et al. An adverse immune-endocrine profile in patients with tuberculosis and type 2 diabetes. Tuberculosis. 2016;101:95-101. doi:10.1016/j.tube.2016.09.001.
4. Bottasso O, Bay ML, Besedovsky H, Del Rey A. The immuno-endocrine component in the pathogenesis of tuberculosis. Scand J Immunol. 2007;66(2-3):166-175. doi:10.1111/j.1365-3083.2007.01962.x.

Study of nitric oxide synthases in endothelial cells during infection with mycobacteria

María de los Angeles Hernández Contreras[1], Alfredo Leaños[2], Reynaldo Tiburcio[3], Arturo Ortega[3], Julieta Luna[1]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
[2]Instituto Mexicano del Seguro Social, Mexico City, Mexico
[3]CINVESTAV-IPN, Mexico City, Mexico
hernandezangeles10@hotmail.com; alfredolm@yahoo.com; rtiburcio@cinvestav.mx; aortega@cinvestav.mx; julietalunah@hotmail.com

Keywords: Bacteria, Endothelial Cells, Infection, Nitric Oxide Synthase, Tuberculosis

Tuberculosis is still one of the top leader infectious diseases in the world, in spite of the vast amount of knowledge gained in the last decades. Endothelial cells are prone to viral infections, and less common to bacterial infections. Mycobacteria can infect several cell types including macrophages, lung epithelial cells, fibroblasts, and endothelial cells among others. We previously reported that endothelial cells are infected by mycobacteria of different virulence including Mycobacterium tuberculosis, Mycobacterium abscessus and Mycobacterium smegmatis. These bacteria enter into the endothelial cells by macropinocytosis or another not well established endocytic process. During mycobacterial infection endothelial cells produce nitric oxide (NO) in response to mycobacterial infection, in some cases NO levels are too high. Endothelial cells constitutively present the endothelial nitric oxide synthase eNOS or NOS3, which under different cell stimuli is activated, producing NO right away. NO is also produced by other nitric oxide synthases: the neuronal synthase nNOS or NOS1 and the inducible synthase iNOS or NOS2. So far the role of the different nitric oxide synthases responsible of NO production by infected endothelial cells is unknown. Therefore in this study we analyzed the expression of these isoforms after during an infection with mycobacteria of different virulence. As a research model, we establish the infection of the endothelial cell line HUVEC-EA hy926; endothelial cells were infected with M. tuberculosis, M. smegmatis and M. abscessus. The different NOS isoforms were identified by immunofluorescence and western-blot. Confocal images demonstrated that in the basal stage, only the eNOS isoform was present and for the different mycobacterial infection the other isoforms were also expressed. Specifically, in the case of M. smegmatis infection, an early production of iNOS but specially nNOS was found, while an increase in the expression of eNOS was observed along the kinetics of the infection. The infection caused by M. abscessus, induced the lowest expression of NOS isoforms; at early stages

of the infection the three isoforms were produced with a significant overexpression of nNOS up to the 24 hr post-infection, after this time, a low production of the three synthases was observed up to the end of the observation. Contrary to M. abscessus infection, M. tuberculosis infection induced the highest expression of NOS isoforms; iNOS and nNOS were present from early times of infection and their expression was maintained throughout the infection, for the case of eNOS, an increase of up to 3 times the levels observed in the basal stage were reach and maintained all throughout the infection. This results were corroborated by western-blot and the trend was similar to the one described by immunofluorescence. Endothelial cells during mycobacterial infections produce NO by synthases others than eNOS. Our results demonstrate that endothelial cells have the ability to differentially activate the isoforms of nitric oxide synthases in the presence of mycobacterial infection. NO produce by NOS may contribute to the control of mycobacterial load, but overproduction of this molecule could be also detrimental for endothelial cell homeostasis.

Potential immunogenic role of synthetic peptides derived from ZIP transporters of Trichomonas vaginalis

Loremy Yehudí Cauich Suárez, Julio César Torres Romero, Julio César Lara Riegos, Mario Alberto Ramirez Camacho, Victor Ermilo Arana Argáez

Autonomous University of Yucatan, Mérida, Mexico
loremy.cs@gmail.com; julio.torres@correo.uady.mx; julio.lara@correo.uady.mx; mario.ramirez@correo.uady.mx; victor.arana@correo.uady.mx

Keywords: Antibodies, Immunization, Interferon-gamma, Peptides, Trichomonas vaginalis, ZIP transporter

Introduction: Trichomonas vaginalis is the etiological agent of human trichomoniasis, the most common non-viral sexually transmitted infection (STI) worldwide. Although drug treatment is available for this infection, but there is still no vaccine for control or prevention. Therefore, the identification of new and potent immunogens in T. vaginalis, which can contribute to the development of an immunodiagnostic tool and/or a vaccine against this parasite, is necessary. In this regard many studies have focused on searching for the epitopes of trichomonad antigens that are capable of inducing protective immunity responses against this parasite. Recently, we characterized the transcriptional expression of 8 genes encoding putative ZIP family members (TvZIP), that could be involved in the transport of Zn and Fe in T. vaginalis. The component of several metal transporters from diverse microorganisms have been reported as immunogenic proteins. However, there are no published studies relating to the antigenic characterization of trichomonad metal transporters, using synthetic peptides. Here we report the identification and immunogenicity of synthetic peptides derived from TvZIP transporter sequences.

Aim: Assess the immunogenicity of synthetic peptides derived from TvZIP transporters in a murine model.

Experimental Strategies: The peptides were predicted by bioinformatic programs combining hydrophilicity, flexibility, surface probability, secondary structure and antigenic index parameters of the amino acid sequence of the TvZIPs transporters. Synthetic peptides were immunized in BALB/c mice for 45 days. After immunization, antibody

levels in mice serum and cytokines from the supernatant of macrophages and from co-culture systems were measured by ELISA.

Results: Supenatants from co-culture of stimulated macrophages and TCD4+ from immunized mice shown increased levels of INF-γ when compared with negative control (naive LTCD4+ and unestimulated macrophages). Increased IgG antibody production were detected in immunized groups.

Conclusions: TvZIPs are capable of induce production of IgG antibodies and an increased secretion of INF-γ by TCD4+ lymphocytes from immunized mice. These results suggest the immunogenic potential of TvTRPV from T. vaginalis through recognition and memory generation.

The interplay between CD43, CD28 and the TCR signals can lead to different activation outcomes

Monserrat Alba Sandoval Hernandez[1,2,3], Erika Melchy[1,3], Estefanía Alemán[1,2,3], Jorge Carneiro[4], Nora A Fierro[5], Ivan Imaz-Rosshandler[6], Ernesto Pérez-Rueda[7], Yvonne Rosenstein[1,3]

[1]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
[2]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[3]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico
[4]Instituto Gulbenkian de Ciência (IGC), Oeiras, Portugal
[5]Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
[6]University of Cambridge, Cambridge, United Kingdom
[7]Instituto de Investigaciones en Matemáticas Aplicadas y en Sistemas, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
monseinm@ibt.unam.mx; melchy@ibt.unam.mx; steph@ibt.unam.mx; jcarneir@igc.gulbenkian.pt; noraalma@gmail.com; ii236@cam.ac.uk; ernesto.perez@iimas.unam.mx; yvonne@ibt.unam.mx

Keywords: modulation, Inflammatory profile, CD28 co-stimulation, CD43 sialomucin co-stimulation, human T lymphocytes activation

The co-receptor molecule CD43 is the major sialylated glycoprotein expressed on the surface of hematopoietic cells. It is very abundant on T lymphocytes, occupying approximately 20% of the cell surface, and because of its amino-acid composition and glycosylation, it adopts the form of a rigid antenna that protrudes 45nm out of the cell membrane. We reported previously that the timing when a T cell senses the TCR signals relative to those of a co-receptor molecule such as CD43 determines the cell's ability to be activated. If CD43 engagement takes place before antigen recognition, cells enter into a proliferative program, as opposed to cells first activated through the TCR. This study aimed to extend these observations to CD28, considered as the master accessory molecule and to compare the response of human T lymphocytes stimulated through the TCR and CD43 and/or CD28 following different schemes of activation. The expression of cytokines/chemokines/growth-factors specific of different Th profiles as well as the expression level of activation markers such as CD40L, CD25, PD1 and FASL were evaluated following activation of human T lymphocytes with CD43 and/or CD28 simultaneously, before or after activation through the TCR.

Depending on when the cells sense the information transduced by CD43 or CD28 vis-à-vis of the TCR signals, the sets of proteins secreted and their relative amounts were different, significantly modulating the type of immune response. Specifically, engaging CD43 (or CD28) simultaneously or before TCR ligation resulted in an activation program leading mostly to a pro-inflammatory profile and potentially favoring clonal expansion, whereas stimulating the cells through the TCR before ligating CD43 or CD28 resulted in poor IL-2 production and low levels of cytokines and chemokines. The activation program was very similar when engaging CD43 or CD28 simultaneous or prior to the TCR. However, when considering the intensity of the response, the amplitude of the signal delivered by CD28 was considerably more elevated than that provided by CD43. Furthermore, in the tripartite scheme of activation, whether CD43 signals were sensed before or at the same time than those of CD28 and the TCR, the CD43-mediated signals attenuated the amplitude of the response as compared to that of TCRXCD28 activated cells. Altogether, our data highlight a complex interplay between CD28 and CD43 in modulating the TCR signals and the resulting expression pattern of molecules that shape the outcome of an immune response.

Inhibins modulate T cell activation and T effector function differentiation

Sandra Ortega-Francisco[1], Marisol De La Fuente-Granada[1], Roxana Olguín-Alor[1], Laura Bonifaz[2], Eduardo García-Zepeda[1], Gloria Soldevila[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
amyrisortega@gmail.com; mdelafuente@iibiomedicas.unam.mx; rolguinalor@gmail.com; labonifaz@yahoo.com; garciaze@unam.mx; soldevi@servidor.unam.mx

Keywords: Inhibins, TGF-beta Superfamily Proteins, Th1 Cells, Th17 Cells, TGF-beta, T effector cells

The Transforming Growth Factor β superfamily members participate in the immune system as key regulators of several cellular functions. Our group has previously reported that Inhibins and its molecular pair, TGF-β type III receptor (TβRIII), regulate the development of T cells within the thymus. We recently reported that TβRIII is upregulated after TCR stimulation, in parallel to other early activation markers. In contrast, natural and induced Tregs downregulated TβRIII in association with Foxp3 upregulation. However, the intrinsic role of Inhibins in T cell activation and differentiation is currently unknown. Here, we describe for the first time that T cells are able to produce Inhibin A after a polyclonal TCR stimulation (anti-CD3 plus anti-CD28), showing a peak of Inhibin A secretion at 96h after the stimulation. As Inhibins have been shown to antagonize Activin mediated functions in some cell types, we investigated if the absence of Inhibins may results in enhanced Activin producitons. Interestingly, Inh-/- T cells produced normal levels of Activin A, indicating that changes in the functional responses of these cells result of intrinsic defects in Inhibin production and not to differences in Activin concentrations. Interestingly, Inhα -/- naïve T cells showed lower expression of activation markers (CD25, CD44, CD69) after TCR stimulation, including TβRIII, recently reported by our group as an early activation marker, at all time point evaluated (12h to 96h), compared with their wild type counterparts. Moreover, Inhα -/- naïve T cells showed a significant decrease, of almost 50%, in Th1 differentiation under skewing conditions (10 ng/ml of IL-12 and 10 μg/ml of anti-IL-4) that correlated with lower levels of activation. Furthermore, when we evaluated the differentiation towards TGFβ-dependent lineages, we observed an increase in Th17 as well as Treg differentiation in Inhα-/- naïve T cells compared to wild type. Specifically, the percentage of Th17 differentiation increased 1.6 times, under optimal activation and skewing conditions (20 ng/ml of IL-6, 10 ng/ml of IL-23, 1.5 ng/ml of TGF-β, 10 μg/ml of anti-IL-4 and 10μg/ml of anti-IFN-γ). Moreover, the number of in vitro differentiated

Tregs increased 1.5 times under conditions of suboptimal activation in the presence of 1ng/ml TGF-β in association with an increment in Inhα -/- T cell survival which was also evidenced under Th1 and Th17 skewing conditions. These data indicate that Inhibins play an intrinsic role during T cell activation and may modulate T effector cell differentiation during the immune responses.

Clinical relevance of the GAL1-glycan axis in multiple sclerosis

Santiago Patricio Mendez Huergo[1], Tomas D'alotto-Moreno[1], Mauricio Franco Farez[2], Jorge Correale[2], Karina Valeria Mariño[1], Marta A Toscano[1], Gabriel Adrián Rabinovich[1]

[1]Instituto de Biología y Medicina Experimental (IBYME), Buenos Aires, Argentina
[2]Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia, Buenos Aires, Argentina
santiago.mendezhuergo@gmail.com; tomasdalotto@gmail.com; mauriciofarez@gmail.com; jcorreale@fleni.org.ar; kvmarinosp@gmail.com; martalitos@yahoo.com.ar; gabyrabi@gmail.com

Keywords: Autoimmune Diseases, Glycosylation, Multiple Sclerosis, regulatory T cells, Galectin-1

Glycosylation is a highly regulated process capable of modulating the fate and function of T cells, key players in autoimmune diseases including multiple sclerosis (MS), rheumatoid arthritis and inflammatory bowel disease. MS is a demyelinating disease of the central nervous system (CNS) that affects approximately 2.5 million people worldwide. In the present work, we aim to study the T-cell surface glycome in regulatory (Treg) versus effector T (Teff) cells and to capitalize on glycan-galectin interactions for the design of novel therapeutic strategies for autoimmune CNS inflammation. We found that treatment with Galectin-1 (Gal1) a lectin that binds Gal beta1,4 GlcNAc (N-acetyl-lactosamine -LacNAc-) present mainly in N- and O-glycans, ameliorates the clinical symptoms of mice bearing experimental autoimmune encephalomyelitis (EAE) ($p < 0.05$), an animal model of MS. Immunologically, Gal1 dampens Th1 and Th17 responses while enhances the Treg cell compartment ($p < 0.01$), both at the CNS and draining lymph nodes. We found that Tregs but not Teff cells were protected from Gal1-induced apoptosis ($p < 0.001$) through differential glycosylation of cell surface receptors. Mechanistically, we observed by mass spectometry that Tregs and Teff cells showed differential levels of complex N-glycan branching and alpha 2,6 sialylation (α2,6SA) of their cell surface glycoproteins. While Tregs presented lower levels of Gal1-binding structures and higher levels of α2,6 sialylated ligands on complex N-glycans, Teff cells showed higher frequency of LacNAc structures and lower levels of α2,6 sialylated ligands. Furthermore, we studied the clinical relevance of Gal1 and its specific glycosylated ligands on T cells from MS patients. In a first cohort, we found that during the remitting phase of the disease, MS patients show lower levels of circulating Gal1 when compared with healthy donors ($p < 0.001$). These findings were confirmed in a second, independent cohort. Additionally, we observed that during a relapse, circulating levels of Gal1 were even lower than those detected during the remitting phase ($p < 0.001$). Moreover, we identified a subpopulation of CD4+ T cells that arised at this stage and was absent during the remitting phase and in healthy

donors, characterized by low levels of cell surface α2,6SA ($p < 0.001$). This T-cell subpopulation showed phenotypic features of Teff cells and displayed high sensitivity to Gal1-induced apoptosis ($p < 0.001$). Our findings emphasize the clinical relevance of the Gal1-glycan axis in MS patients and, together with our pre-clincial observations, provide strong evidence supporting a Gal1-based therapeutic strategy in autoimmune CNS inflammation.

The interaction between CD43 and Mycobacterium tuberculosis' chaperonin "Cpn60.2" leads to an inflammatory response based on IFN-γ and TNF-α in T cells

Den Alejandro Alvarado[1,2,3], Álvaro Torres[1,2,3], Estefanía Alemán[1,2,4], Cristina Parada[5], Clara I Espitia[5], Rogelio Hernández[6], Yvonne Rosenstein[1,2]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico
[2]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
[3]Posgrado en Ciencias, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[4]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[5]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[6]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
denale@ibt.unam.mx; avery@ibt.unam.mx; steph@ibt.unam.mx; cristinaparada2004@yahoo.com.mx; espitia@biomedicas.unam.mx; rogelio.hernandezp@incmnsz.mx; yvonne@ibt.unam.mx

Keywords: Cytokines, T cells, TNF-a, IFN-g, CD43, Cpn60.2

Mycobacterium tuberculosis (Mtb) is the etiologic agent of tuberculosis, a prevalent disease that mainly affects lungs and claims the lives of approximately 1.4 million people worldwide each year. Mtb arrives at alveoli after aerosol droplets exhaled by individuals with active tuberculosis are inhaled. In the alveoli, Mtb interacts with alveolar macrophages and dendritic cells as virulence factors, lipoproteins, chaperonins and other proteins of Mtb, known as Pathogen Associated Molecular Patterns (PAMPs), are recognized by Pattern Recognition Receptors (PRRs) expressed by macrophages and dendritic cells. Among these PRRs are the Toll-Like Receptors (TLRs), CD14, mannose receptors and more recently, the CD43 sialomucin. CD43, also known as sialophorin or leukosialin, is a highly sialylated glycoprotein expressed in all hematopoietic cells except erythrocytes.

The activation of macrophages and DCs resulting of the interaction between PRRs and Mtb-derived PAMPs leads to the release of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ). IFN-γ enhances the functions of other immune cells, leading to efficient containment and clearance of Mtb and TNF-α is necessary to enable cell-intrinsic mechanisms for Mtb control. Murine models deficient for those proteins have proved that they are both essential for the establishment and maintenance of granulomas wherein multiple immune cells participate

in containing and preventing further spreading of Mtb. Additionally, activation licenses macrophages and DCs as Antigen Presenting Cells (APCs) that migrate to the nearest lymph node and prime T cells, resulting in their differentiation into Th1, Th17, and Th2 cells. Th1 cells are the primary producers of IFN-γ. Th17 cells secrete IL-17, a cytokine that promotes the production of inflammatory cytokines and the recruitment of monocytes and neutrophils to the granulomas.

CD43, also known as sialophorin or leukosialin, is a highly sialylated glycoprotein expressed in all hematopoietic cells except erythrocytes. CD43 has been reported to interact with Cpn60.2, a chaperonin essential for mycobacterial growth. When localized on the outer cell wall, Cpn60.2 functions as an adhesin, favoring the interaction between the macrophages and the mycobacteria, through CD43. Our laboratory recently reported that the interaction between CD43 and the multimeric Cpn60.2 leads to the production of TNF-α in both human and murine macrophages. Preliminary In vivo experiments have shown that CD43 -/- mice fail to produce IFN-γ and enhanced IL-17 production when challenged with Mtb, underscoring the importance of CD43 and T cells in the containment of Mtb. The present study aimed to evaluate the impact of the CD43-Cpn60.2, -BCG or -Mtb interaction on T cell function. Data obtained with Jurkat cells expressing normal levels of CD43, cells where CD43 expression level was inhibited by RNA interference or cells expressing a form of CD43 lacking the intracellular domain as well as T lymphocytes isolated from CD43 deficient mice, or their wild-type mice counterparts will be discussed. Overall, data indicate that CD43-mediated signals modulate inflammatory responses in T cells, in response to Cpn60.2 or –BCG stimulation.

The CD43 Intracelular domain regulates Ag-specific T cells responses

Erika Isabel Melchy-Perez[1,2], Ivan Carranza-Morales[1,2,3], Angel Francisco Flores-Alcantara[1,2], Gustavo Pedraza-Alva[1], Yvonne Jane Rosenstein-Azoulay[1,2]

[1]National Autonomous University of Mexico, Cuernavaca, Mexico

[2]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico

[3]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

melchy@ibt.unam.mx; bio.carr.mol.iv@gmail.com; alcantar@ibt.unam.mx; gustavo@ibt.unam.mx; yvonne@ibt.unam.mx; yvonne@ibt.unam.mx

Keywords: T cells, Accessory molecules, Antigen-specific response, CD43, OT mice

The immune response is an adaptative mechanism that modulates the relationship between the external and the internal environment, recognizing unusual agents or molecules in the organism and mounting the most appropriate response to ensure the integrity of the individual. T cells (CD4+ and CD8+) are among the principal components of this elaborate system. The T cell receptor (TCR) recognizes the MHC-Ag complex as presented by antigen-presenting cells (APCs), and sets in motion the activation program of the cell. However, full activation is only achieved when accessory molecules and their counter-receptors present on the APCs provide enough information for the cells to differentiate and commit to an effector function. Without the information provided by the accessory molecules, cells fail to execute the cell activation program entirely and instead acquire an anergic state. The sialomucin CD43 is an accessory molecule. This heavily sialylated glycoprotein is a type I transmembrane protein expressed by all the hematopoietic lineage cells but erythrocytes. CD43 participates in a wide range of cellular functions such as modulation of cell adhesion, cell survival, and cell cycle entry. Recent studies of our laboratory, evidenced that CD43-dependent signals contribute to the activation of T lymphocytes. Notably, when combined with the TCR signals, CD43 signals inhibit the c-Cbl and Cbl-b negative function, increasing the duration and intensity of the signals of the TCR and lowering the threshold for activation. These effects are mediated by the highly conserved cytoplasmic domain of CD43 since deletion of the cytoplasmic domain abolishes the co-receptor functions of CD43. To better characterize the accessory molecule function if CD43, we generated transgenic mice expressing a mutant form of the protein lacking the cytoplasmic region (CD43ΔIC-GFP) under the control of the distal promoter of Lck, thus favoring expression of the transgene in peripheral T lymphocytes. To evaluate the role of the CD43ΔIC-GFP form

of CD43 in vivo, on an antigen-specific response, we crossed these mice with OT-I or OT-II transgenic mice. Overall, our results indicate that CD43ΔIC-GFP functions as a dominant negative molecule, dampening antigen-specific T cell response, but that the mechanism through which this happens is not the same for CD4+ and CD8+ T lymphocytes.

Funded by PAPIIT/DGAPA, UNAM and CONACYT, MEXICO.

Long-term culture of human resting CD4+ T cells: homeostatic cytokine concentration-dependent expression of activation markers and proliferation in memory cells

Isabel Ruelas-Galindo, Leonor Huerta

Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
isargol_06@hotmail.com; leonorhh@biomedicas.unam.mx

Keywords: IL-7, homeostatic proliferation, IL-15, Activation markers, human resting CD4+ T cells

Background: Circulating resting CD4+ T cells comprise the naïve and memory cell populations, which have a long-life span and an intermittent-dividing state without changes of their phenotype, a process called homeostatic proliferation. The main factors allowing survival and proliferation are the contact with homeostatic cytokines and/or low-affinity interactions with peptide/MHC expressed on the surface of other cells. It has been demonstrated that the prolonged survival and the homeostatic proliferation in vivo of human naïve CD4+ T cell population is induced by peripheral mechanisms and thymopoiesis. However, during age-associated thymic involution, the preservation of the naïve pool is maintained mainly by peripheral mechanisms, being IL-7 the principal inductor of survival by promoting the expression of the prosurvival molecules Bcl-2, Bcl-xL and Mcl-1 1,2. One of the main sources of IL-7 are the fibroblastic reticular cells in the lymph nodes, while in the bone marrow and thymus, the IL-7 is produced by stromal cells 3. Naïve cells produced in the thymus are released to the periphery, where they recirculate through the blood and the secondary lymph organs, and compete with each other for survival and homeostatic cytokines. On the other hand, the in vivo maintenance of the memory CD4+ T cell pool is not completely understood, and it is possible that different mechanisms contribute to the survival and the homeostatic turnover of different memory subpopulations. Memory cells circulate throughout the body, and they can be found in non-lymphatic tissues or in secondary lymphatic organs. It is reported that memory CD4+ T cells require IL-7 and IL-15 4,5, and it is not well established if they actually need the contact with peptide/MHC like naïve cells for homeostatic proliferation 6,7,8. IL-15 is produced by a variety of cells, such as antigen-presenting cells (APCs), stromal and epithelial cells 9.

Most of the evidence about the survival and homeostatic proliferation of mouse and human resting CD4+ T cells has been obtained within limited times of culture (24 or 48 hours), and there are no reports about the maintenance of human resting CD4+ T cells for longer periods in vitro.

Objective: Here, we determined the in vitro conditions for the long-term maintenance of purified naïve and memory human CD4+ T cells in the presence of variable concentrations of homeostatic cytokines. Proliferation, apoptosis, and expression of early activation markers (CD25, CD54, and CD69) were determined. Culture conditions excluded contact with other cells and fetal serum growth factors.

Materials and Methods: Peripheral blood mononuclear cells (PBMCs) were obtained by centrifugation in a Ficoll Hypaque gradient. From PBMC, naïve or memory CD4+ T cells were purified by negative selection. Recently purified cells were cultured in the fetal serum-free AIM-V medium (Gibco) plus 5 % of AB adult human serum in 48-well plates. For memory cells, different concentrations of the IL-7 and IL-15 cytokines (1, 10 and 100 ng/ml) were added and cells were incubated at 37°C with 5% of CO2 for the indicated times. For naïve cells only 20 ng/ml of IL-7 was used. The expression of CD4, CD45RA or CD45RO, the activation markers CD25, CD54, CD69 and the marker of proliferation Ki67, was analyzed by flow cytometry using a six-color assay. Cell death was determined by trypan blue exclusion and apoptosis by an Annexin V/7-AAD assay on CD45RO+ CD4+ cells using a four-color assay. Cells were analyzed with an Attune Nxt Acoustic Focusing Cytometer at variable culture times.

Results: Survival of memory CD4+ T cells required the addition of both IL-7 and IL-15 cytokines, showing a >80% viability after 8 days and low level of apoptosis, in the presence of 10 or 100 ng/ml of these cytokines. As the concentration of cytokines raised (separated or combined), the expression of activation markers and proliferation increased. At the higher concentration used (100 ng/ml), between 30 to 40% of cells expressed low levels of the activation markers CD25, CD54, CD69 and the proliferation marker Ki67. No expression of activation markers or proliferation was observed with lower cytokine concentrations (10 ng/ml).

Naïve CD4+ T cells survived 30 days in the presence of 20 ng/ml of IL-7, whereas maintaining a constant viability (>95%). None of the activation markers was expressed and no proliferation was observed.

Conclusions: We established a methodology for the long-term culture of purified resting human CD4+ T cells in the absence of fetal bovine serum. Incubation with moderated concentrations of memory cells with IL-7 and IL-15 did not induced the expression of activation markers or proliferation. We observed expression of the activation markers and proliferation in a subpopulation of memory cells only at the higher concentration of both cytokines (100 ng/ml). These observations indicate that memory

CD4+ T cells are susceptible to activation by high concentrations of the IL-7 and IL-15 cytokines. The activated population deserves further characterization. On the other hand, naïve cells can be cultured several weeks in the presence of 20 ng/ml of IL-7, keeping high viability without the expression of activation markers.

Culture conditions stablished in this work will be further applied to the study of factors influencing the infection of resting CD4+ T cells by the HIV-1.

Zinc supplementation and its impact on the production of Interleukin 21

Ana Esther Aguilar Cardenas, Nelly Rodríguez Díaz, Maria Guadalupe Espejel Maya

Faculty of Chemistry, National Autonomous University of Mexico, Mexico City, Mexico
nelly.r.di@gmail.com; dranitae@gmail.com; gpespejel@hotmail.com

Keywords: Mice, Nutritional Status, Zinc, IL-21, perinatal stages

Introduction: Interleukin 21 (IL-21) is a cytokine produced by Th17, follicular T and NKT lymphocytes. It is involved in different diseases related with the inmune system due to its ability to promote the production of autoantibodies. Given the wide variety of targets to be modulated and its variety of pleiotropic actions, this and its receptor are attractive targets for therapeutic manipulation. It is known that the consequences of zinc deficiency affect lymphopoiesis, practically all types from mature immune cells, the production of cytokines and the polarization of subsets of helper T lymphocytes. This study aimed to evaluate the effects of zinc supplementation on the production of IL-21.

Material and Methods

Experimental Design

BALB/c AnN mice 22 g, n=50 were divided into 2 main groups. They were fed ad libitum (Lab Diet, ProLab® 2500 RMH 5P14, USA). To determine how the consumption of zinc affects the production of IL-21 at critical stages of development, a murine model of supplementation was used. The experimental animals received water with 500 or 180 mg/L of zinc (Zn acetate (Zn (C2H3O2 • 2H2O)) Mallinckrodt® CAS 5970-45-6) ad libitum, meanwhile the control animals only received zinc from the oval pellets.

Zn was administered from mating to after two weeks the male was withdrawn, and the dams continued with supplementation until their offspring F1 finished their lactancy period (3 weeks), or until 42 days age (6 weeks).

In vivo stimulation with LPS

Dose-response curve of lipopolysaccharide and the production of IL-21 in mice were performed, with concentrations of 1, 3, 5, 7.5 and 10 mg/kg LPS.

Groups were stimulated 3 mg/kg of lipopolysaccharide (Sigma-Aldrich®, E. coli O111: B4, Cat: L2630) intraperitoneal in 200 µL of sterile saline solution, 24 and 48 hours before to induce the production of IL-21 in serum.

Collection of samples

The blood samples were obtained by retro-orbital bleeding, these were collected in 2.0 mL microcentrifuge tubes (COSTAR®, Cat: 3213), then to coagulate at 35 ° C for 30 minutes and centrifuged at 5000 rpm for 20 minutes (Centrifuge IEC Centra-M2). Recovered the serum. Samples were freezed at -60 ° C.

Detection of murine IL-21 by double antibody enzyme immunoassay (EIA)

Antibodies (PeproTech® Cat. 900-M368) were used, antibodies anti-IL-21 were produced in rabbit and the detection antibody was biotinylated for the identification of murine IL-21 in serum, as cromogen ABTS was used (SIGMA® Cat. A3219). The absorbance was measured to 405 nm in a microplate reader (Behring EL, Microstrip Reader).

Statistical analysis

All experiments were performed in triplicate, and the results were expressed as the mean ± standard deviation. GraphPad Prism® program version 7.03 was used to analyze the data. ANOVA test was applied to determine if there were differences among the treatment groups (a p-value less than 0.05 was considered significant). Tukey's method was used to multiple comparisons.

Results: Results of the evaluation of IL-21, treatment of 6 weeks with zinc: There is a clear significant (with a drop of 40% to compare to control) in cytokine levels when the zinc in the supplementation is 180 mg/L. The previous administration with LPS promotes an increase in the production of IL-21 in the positive control from 1240 to 1400 pg/mL respect to the control. In the supplement group Zn 500 mg/L the result compared to the control. The treatment was in the stages of pregnancy and lactation. The results were analyzed by one-way ANOVA. P> 0.05 (Fig. 1).

IL-21 production, 9-week Zn treatment: Treatment was maintained during pregnancy, lactation and after weaning. There is a clear decrement of 30% respect to the control in cytokine levels when supplemented with 180 mg/L of Zn. A slight increment (5%) is also showed in the 500 mg/L zinc group with or without LPS. The results were analyzed by one-way ANOVA. * P <0.05 and Tukey's method (Fig. 2).

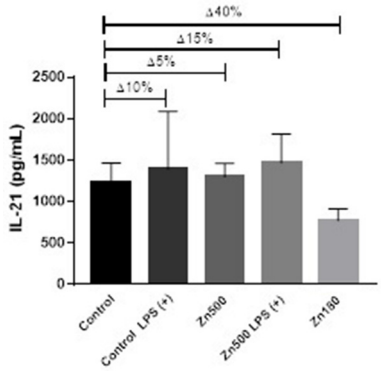


FIGURE 1: Results of the evaluation of IL-21, treatment of 6 weeks with zinc.

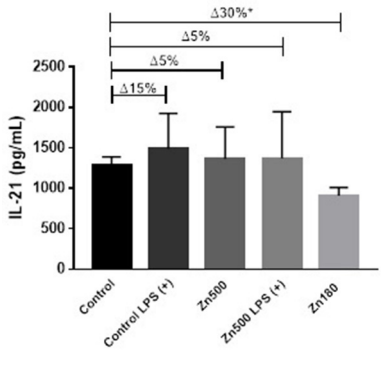


FIGURE 2: IL-21 production, 9-week Zn treatment

Discussion and Conclusion: Our study showed that interleukin 21 levels production with zinc supplementation of 500 mg/L, and decrease with zinc of 180 mg/L, 6 weeks treatment results were noticeable. Our results showed that the production of the cytokine depends the concentration of zinc administered and the stage of development. This study provides evidence that confirms the high degree of regulation carried out by immune cells in zinc homeostasis. The moderate differences between the concentrations of IL-21 using zinc as a supplement indicates a tight regulation of this metal by immune cells. Even an immunostimulation is carried out, the differences founded between the experimental groups are not severe on health.

The role of IgC-like domain during CRTAM-Necl2 interaction

Juan Carlos Barragan-Galvez[1,2], Jose Luis Maravillas-Montero[3], Vianney Francisco Ortiz-Navarrete[2]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
[3]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
jcbarragang@gmail.com; jlmaravillas10@gmail.com; vortiz@cinvestav.mx

Keywords: Receptors, Cell Surface, Adhesion molecules, cell-cell adhesion, CRTAM, NECL2

CRTAM is a transmembrane protein belonging to the immunoglobulin superfamily that is expressed in activated T lymphocytes and constitutively in epithelial cells, composed of a variable (IgV) and constant (IgC) immunoglobulin-like domain on the extracellular region, and interacts through the V-domain with Nectin-Like 2 (Necl2), ligand that is expressed constitutively on the dendritic cell surface (DC) and epithelial cells. However, the role of the constant Ig-Like domains during the interaction of CRTAM-Necl2 have not been studied.

The extracellular region of CRTAM and Necl2 were cloned and expressed in bacterial system. The recombinant proteins were purified for affinity chromatography and subjected to gel filtration, and the kinetic parameters of the interactions were measured by Surface Plasmon Resonance.

The results show that the IgC domain of CRTAM is present in oligomeric forms in solution, and the affinity of adhesion of CRTAM-Necl2 is of 2.16 nM, while the affinity decreases to 0.9 μM when the IgC domain is absent. Interaction assays also demonstrated that IgC domain interacts with Necl2. This suggest that the IgC domain is involved in the avidity during adhesion of both molecules.

Sepsis-induced chronic inflammation impairs CD8 T cell effector function through an epigenetic mechanism

J. Alejandro Cisneros-Segura[1,2], Noe Rodriguez-Rodriguez[2,3], Giovanna Flores-Mendoza[2,4], Sergio Iván Valdés-Ferrer[2], Florencia Rosetti[2], José Carlos Crispín[2]

[1]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[3]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[4]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
alejandro.jcs25@gmail.com; noahrodriguez2@gmail.com; gionisse@hotmail.com; sivaldes@gmail.com; florencia.rosettis@incmnsz.mx; carlos.crispina@incmnsz.mx

Keywords: Immunosuppression, Sepsis, IFN-gamma, CD8 T cell, lncRNA (long non-coding RNA)

Sepsis is a complex clinical syndrome caused by exuberant and uncontrolled activation of the innate immune system that entails high morbidity and mortality. Sepsis represents the pathologic common final pathway of a relatively large number of inflammatory triggers, usually severe infections. The development of multi-organ failure in patients with sepsis remains a major cause of death in intensive care units. Unfortunately, patients who survive the acute phase of sepsis commonly develop a chronic inflammatory syndrome characterized by hematopoietic dysfunction, neurological deterioration, and immunosuppression. This syndrome has a poor prognosis with mortality rates as high as 80% at 5 years. Infections represent an important concern in these patients and reactivation of latent viruses, in particular cytomegalovirus and herpes simplex virus, is commonly observed.

The aim of this work was to develop a murine model that would enable us to study the mechanisms through which sepsis affects the adaptive immune system, in particular CD8+ T cell effector function. To this end, we developed a two-phase model in which polymicrobial sepsis was followed by infection with Listeria monocytogenes, an intracellular bacterium controlled by CD8+ cells, in order to analyze the consequences of sepsis on the CD8+ T cell behavior.

We induced abdominal sepsis (cecal ligation and puncture; CLP) in wild-type and OT-I mice. Thirty days later, OT-I cells were isolated from septic (or control) mice and were adoptively transferred into septic or control WT mice: (a) control OT-I cells in

control recipient mice; (b) septic OT-I cells in control recipient mice; (c) control OT-I cells in septic recipient mice; (d) septic OT-I cells in septic recipient mice. One day after OT-I T cell adoptive transfer, mice were infected with ovalbumin-expressing L. monocytogenes (LM-OVA). Bacterial clearance and CD8 effector function (i.e. OT-I cell expansion, cytokine production, cytotoxicity) was analyzed.

Our experimental approach allowed us to distinguish the effects that sepsis imposed directly on CD8+ T cells from those exerted on other components of the immune system. OT-I cells transferred into septic mice proliferated less than OT-I cells transferred into control mice. This phenomenon was associated to a significant decrease in the number of CD11c+ dendritic cells in the spleens of septic mice. When we compared the behavior of OT-I cells isolated from septic and control mice and transferred into control recipients, we observed that OT-I cells obtained from a septic environment exhibited a significant impairment in cytotoxic function and in the production of IFN-gamma. To determine whether the impaired effector capacities represented the result of prolonged CD8 T cell activation during the chronic inflammatory environment, we quantified cell division in OT-I T cells exposed to 30 days of abdominal sepsis. CFSE dilution was similar in OT-I cells transferred into septic and control mice, indicating that the effector impairment did not result from chronic activation. We compared the expression profile of effector function-associated genes in septic and control OT-I cells and found that expression of Ifng and Runx3 were abrogated in cells isolated from septic mice. Interestingly, Nest, a long-noncoding RNA that promotes the transcription of Ifng through the local recruitment of the Trithorax complex and the facilitation of local H3K4me3, was absent in OT-I cells derived from septic mice.

Here, we describe a model that allows us to study the effects of chronic inflammation on CD8+ T cell effector function and behavior. Our results demonstrate that sepsis has widespread effects on several cells of the immune system, but they also show that CD8+ T cells are directly affected by the chronic inflammatory environment, even in the absence of cognate antigen stimulation. Importantly, we show that the impaired function of CD8+ T cells is imposed at a transcriptional level and is associated to defects in the expression of a lncRNA.

Analysis of activation, proliferation and IL-2 and IFN-γ production in effector and central memory T cells in response to Mycobacterium tuberculosis

Yesenia Brito-Pérez, Iris Citlali Estrada-Garcia, Sergio Antonio Estrada-Parra, Claudia Sandoval-Montes

Instituto Politécnico Nacional, Mexico City, Mexico
brito-perez@hotmail.com; iestrada5@hotmail.com; sestradap07@hotmail.com; claudiaqfb@yahoo.com.mx

Keywords: Th1 Cells, Tuberculosis, IL2, memory T cells, IFN-γ

Tuberculosis is a disease caused by Mycobacterium tuberculosis, which can remain inactive and asymptomatic for years. Early detection is important to prevent the development of the disease. In 10% of infected people, clinical tuberculosis disease develops; 10.4 million new cases occurred worldwide in 2016, from which 1.7 million people died. Cell-mediated immune mechanisms, including Th1 type CD4 T cells and CD8 T cells have been proven to have the capability to kill mycobacteria inside pulmonary macrophages. Following infection or BCG vaccination of human, the antigen-specific T cells response and are differentiated to memory T cells producing IFN-γ, IL-2, and TNF-α in adults with active tuberculosis. In this study, We evaluate the activation, proliferation and production of IFN-γ and IL-2 in the subpopulations of T cells (naïve, effector, effector memory and central memory), activated with antigens from Mycobacterium tuberculosis soluble extract strain H37Rv (MTSE). We obtained peripheral blood mononuclear cells from healthy donors BCG vaccinated which were stimulated with PMA and MTSE during 0, 4, 24, 48 and 72 hours, then the cells were stained with anti-CD3, anti-CCR7, anti-CD45RO, anti-CD38, anti-CD25, anti-CD69, anti-CD127, anti-IFN-γ and anti-IL-2 monoclonal antibodies and analyzed by flow cytometry FACS ARIA III. We found that the percentages of MTSE-specific central memory T cells (CD45RO+CCR7+) expressed immune activation markers CD69+, CD25+ and CD38+ also produce IFN-γ and IL-2 compared to effector memory T cells (CD45RO+CCR7-) and the other subpopulation of the T cells. When analyzed the expression of CD127, a marker of mature T cells, We observed that this protein is expressed in both memory T cells subpopulations. In proliferation assays with CFSE (5(6)-Carboxyfluorescein diacetate N-succinimidyl ester), we analyzed the memory T cells on CD3+CD4+ and CD3+CD8+ T cells subpopulations, founding a higher percentage of proliferating CD4+ central memory T cells when the cells were stimulated with the MTSE and the same trend with CD8+ central memory T cells. In conclusion, there are a higher percentage of central memory T cells activated, proliferating and producing cytokines when are stimulated with the MTSE in healthy people vaccinated with BCG.

In vitro exposure to DDE alters miR-146 expression levels in peripheral blood mononuclear cells

Ivan Nelinho Perez-Maldonado, Angeles Catalina Ochoa-Martínez, Juan Carlos Fernández-Macias

Facultad de Medicina, Universidad Autónoma de San Luis Potosi, San Luis Potosí, Mexico
ivan.perez@uaslp.mx; miaka_naeguino@hotmail.com.mx; jcarlos_fernandez@outlook.com.mx

Keywords: DDT, Inflammation, epigenetics, miR-146, DDE

Introduction: Dichlorodiphenyltrichloroethane (DDT) is a synthetic pesticide that was used widely in 20th century, principally in agriculture (cotton crops) and health campaigns for the control of insect-transmitted diseases (Stapleton, 1998). DDT and their metabolites (DDTs), dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), are lipophilic, resistant to degradation, long-range transportable and tend to bioaccumulate within the food chain (ATSDR, 2002). Recent studies have demonstrated that DDE (the most stable metabolite of DDT) is able to induce a pro-inflammatory condition in immune cells [peripheral blood mononuclear cells (PBMC)] exposed in vitro to that chemical compound (Cárdenas-González et al., 2013). However, the molecular mechanism involved in the inflammatory disorder and its regulation are poorly understood. At this concern, current evidence suggests that epigenetic modifications provide a plausible link between the environment and alterations in gene expression (Li et al., 2015). Epigenetic alterations are inheritable factors involved in regulating gene expression that does not involve a change in the nucleotide sequence (Wolffe and Matzke, 1999). Epigenetic modifications can occur through three mechanisms mainly: DNA methylation, covalent modifications of histones and RNAs interference (RNAi) (Egger et al., 2004); within RNAi, the microRNAs (miRNAs), which in the last decade have been described as an emerging epigenetic mechanisms involved in a large number of cellular processes (Qiu et al., 2012), including inflammation (Guida et al., 2013). Therefore, the aim of this study was to identify epigenetic alterations, specifically miRNAs expression changes (miR-146) in PBMC exposed in vitro to DDE.

Methods: Heparinized blood from 120 healthy women volunteers from San Luis Potosi (aged 20–30 years, never exposed directly to DDT) was used to isolate PBMC by Ficoll-Hypaque density-gradient centrifugation. Then, cells were treated with increasing concentrations of DDE 10, 20, 40, 60 and 80 μg mL-1 (Cárdenas-González et al., 2013). After, Resarzurin assay was performed to evaluate cell viability as described previously (Avila-Alejo et al., 2017). Also, total RNA was isolated using TRizol reagent

in accordance with the manufacturer´s instructions, cDNA was synthetized using TaqMan® MicroRNA Reverse Transcription (RT) Kit according to manufacturer´s instructions. Subsequently, 100 ng of cDNA were amplified using TaqMan® Universal Master Mix and TaqMan® MicroRNA Assay primers for human miR-146. Analysis of expression levels of miR-146 were determined with relative expression method 2-ΔΔCt, using RNU48 as endogenous control for data normalization.

Results and Discussion: Figure 1 (supplementary material) shows the cell viability after PBMC were exposed "in vitro" to increasing DDE concentrations (10, 20, 40, 60 and 80 µg mL-1) at different treatment times (1, 3, 6, 12 and 24 h). The highest concentration of DDE tested (80 µg ml-1) had a drastic effect on PBMC viability at 12 h (63%) and 24 h (< 60%) of exposure. Cell viability was also slight decreased when PBMC were dosed with 60 µg ml-1 DDE at 24 h of exposure (75%) (Fig. 1). However, low doses of DDE (10, 20, and 40 µg mL-1) did not have significant changes in cell viability (> 80%) during all incubation periods, including treatment with 60 µg ml-1 DDE at 3, 6, and 12 h of exposure (Fig. 1). In concordance with the cell viability assay, we performed analysis for the expression of microRNAs. Figure 2 shows expression levels of miRNA-146 after PBMC were exposed at different times (1, 3, 6, 12 and 24 hours) to increasing concentrations of DDE (10, 20, 40, 60 and 80 µg mL-1), a significant increased level of miRNA-146 was observed when PBMC were exposed at 6 and 12 hours (Fig. 2C and 2D) to the highest DDE concentrations tested (60 and 80 µg mL-1) ($p<0.05$). Some of the main targets of miR-146 are interleukin-1 receptor-associated kinase (IRAK1) and TNF receptor associated factor 6 (TRAF6) (O'Connell et al., 2012;

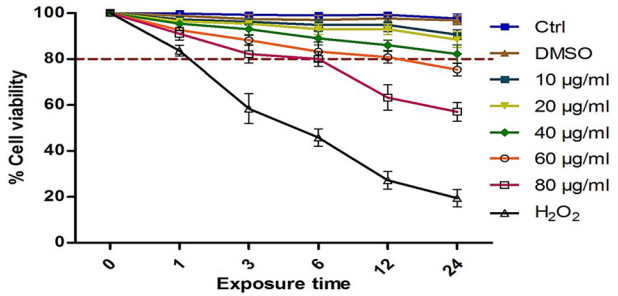


FIGURE 1: Cytotoxic effects of ODE on PBMC assessed by Resazurin reduction assay. PBMC were exposed during 1, 3, 6, 12 and 24 hours to increasing concentrations of DDE (10, 20, 40, 60 and 80 µg mL^{-1}), DMSO (vehicle control) and H_2O_2 (positive control). Cell survival rates were expressed as a percentage with respect to control (unexposed PBMC). Data shows the mean ± S.D. of 120 independent experiments of two replicates each.

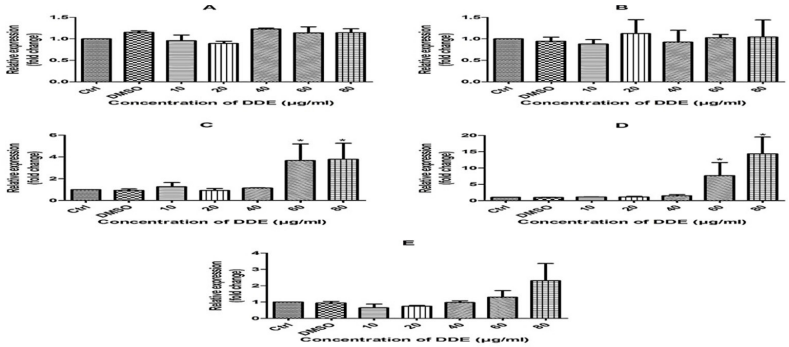


FIGURE 2: Expression levels of miR-146 in PBMC exposed to DDE. Relative expression levels of miRNA-146 at 1 (A), 3 (B), 6 (C), 12 (D) and 24 (E) hours exposed to increasing concentrations of DDE (10, 20, 40, 60 and 80 µg mL^{-1}). The results are plotted normalized to RNU48 and expressed as a fold change ($2^{-\Delta\Delta\delta Ct}$) over the non-stimulated control (Ctrl). Data shows the mean ± SD of 120 independent experiments with two replicates each. * Significantly different from control ($p < 0.05$).

Taganov et al., 2006) which allow it to regulate the inflammatory molecules through downregulation of NF-kB via which is a central inflammatory pathway. Also, recent studies have showed that the expression of miRNA-146 can be altered by inflammatory cytokines (van Scheppingen et al., 2016; Xie et al., 2014). Moreover, several studies suggest that miRNA-146 works as a negative regulator of inflammation. For example, Xi et al. (2013) showed that miRNA-146 inhibits pro-inflammatory cytokine secretion produced through IRAK1 pathway in human gingival fibroblast (HGF) cell line (Xie et al., 2013). Nakasa et al. (2008) reported that miR-146 expression was increased in PBMC in patients with active RA, a disease characterized by inflammation process, (Nakasa et al., 2008). Moreover, Taganov et al. (2006) reported an overexpression of miR-146 in human monocytes in response to a variety of microbial components like lipopolysaccharide (LPS) and proinflammatory cytokines (Taganov et al., 2006). Then, the data above mentioned suggest that up-regulation of miR-146 in PBMC observed in this study, is probable due to a response to increasing inflammatory cytokine levels induced by DDE, as part of a negative feedback loop in order to restrict inflammatory condition. In our knowledge, this is the first study demonstrating an epigenetic effect (miRNAs dysregulation) in PBMC exposed to DDE. However, further studies should be carried out to elucidate the mechanism involved in miRNA change expression and to understand the real significance of these miRNAs dysregulation. In vitro and in vivo studies are planned and will hopefully offer appreciated evidence about the significance of the present results.

REFERENCES

ATSDR, 2002. Toxicological profile for DDT, DDE, and DDD. U.S. Dep. Heal. Hum. Serv.

Avila-Alejo, J.O., González-Palomo, A.K., Plascencia-Villa, G., José-Yacamán, M., Navarro-Contreras, H.R., Pérez-Maldonado, I.N., 2017. Low cytotoxicity of anisotropic gold nanoparticles coated with lysine on peripheral blood mononuclear cells "in vitro." Environ. Toxicol. Pharmacol. 56. https://doi.org/10.1016/j.etap.2017.09.014

Cárdenas-González, M., Gaspar-Ramírez, O., Pérez-Vázquez, F.J., Alegría-Torres, J.A., González-Amaro, R., Pérez-Maldonado, I.N., 2013. p,p'-DDE, a DDT metabolite, induces proinflammatory molecules in human peripheral blood mononuclear cells "in vitro." Exp. Toxicol. Pathol. 65, 661–665. https://doi.org/10.1016/j.etp.2012.07.006

Egger, G., Liang, G., Aparicio, A., Jones, P.A., 2004. Epigenetics in human disease and prospects for epigenetic therapy. Nature 429, 457–463. https://doi.org/10.1038/nature02625

Guida, M., Marra, M., Zullo, F., Guida, M., Trifuoggi, M., Biffali, E., Borra, M., De Mieri, G., D'Alessandro, R., De Felice, B., 2013. Association between exposure to dioxin-like polychlorinated biphenyls and miR-191 expression in human peripheral blood mononuclear cells. Mutat. Res. Toxicol. Environ. Mutagen. 753, 36–41. https://doi.org/10.1016/j.mrgentox.2012.12.018

Li, Q., Kappil, M. a., Li, A., Dassanayake, P.S., Darrah, T.H., Friedman, A.E., Friedman, M., Lambertini, L., Landrigan, P., Stodgell, C.J., Xia, Y., Nanes, J. a., Aagaard, K.M., Schadt, E.E., Murray, J.C., Clark, E.B., Dole, N., Culhane, J., Swanson, J., Varner, M., Moye, J., Kasten, C., Miller, R.K., Chen, J., 2015. Exploring the associations between microRNA expression profiles and environmental pollutants in human placenta from the National Children´s Study (NCS). Epigenetics 10, 793–802. https://doi.org/10.1080/15592294.2015.1066960

Nakasa, T., Miyaki, S., Okubo, A., Hashimoto, M., Nishida, K., Ochi, M., Asahara, H., 2008. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. Arthritis Rheum. 58, 1284–92. https://doi.org/10.1002/art.23429

O'Connell, R.M., Rao, D.S., Baltimore, D., 2012. microRNA Regulation of Inflammatory Responses. Annu. Rev. Immunol. 30, 295–312. https://doi.org/10.1146/annurev-immunol-020711-075013

Qiu, C., Chen, G., Cui, Q., 2012. Towards the understanding of microRNA and environmental factor interactions and their relationships to human diseases. Sci. Rep. 2. https://doi.org/10.1038/srep00318

Stapleton, D.H., 1998. The dawn of DDT and its experimental use by the Rockefeller foundation in Mexico. 1943-1952. Parassitologia 40, 149–158.

Taganov, K.D., Boldin, M.P., Chang, K.-J., Baltimore, D., 2006. NF- B-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proc. Natl. Acad. Sci. 103, 12481–12486. https://doi.org/10.1073/pnas.0605298103

van Scheppingen, J., Iyer, A.M., Prabowo, A.S., Mühlebner, A., Anink, J.J., Scholl, T., Feucht, M., Jansen, F.E., Spliet, W.G., Krsek, P., Zamecnik, J., Buccoliero, A.M., Giordano, F., Genitori, L., Kotulska, K., Jozwiak, S., Jaworski, J., Liszewska, E., van Vliet, E.A., Aronica, E., 2016. Expression of microRNAs miR21, miR146a, and miR155 in tuberous sclerosis complex cortical tubers and their regulation in human astrocytes and SEGA-derived cell cultures. Glia 64, 1066–1082. https://doi.org/10.1002/glia.22983

Wolffe, A.P., Matzke, M.A., 1999. Epigenetics: regulation through repression. Science 286, 481–6.

Xie, Y.-F., Shu, R., Jiang, S.-Y., Liu, D.-L., Ni, J., Zhang, X.-L., 2013. MicroRNA-146 inhibits pro-inflammatory cytokine secretion through IL-1 receptor-associated kinase 1 in human gingival fibroblasts. J. Inflamm. 10, 20. https://doi.org/10.1186/1476-9255-10-20

Xie, Y., Shu, R., Jiang, S., Song, Z., Guo, Q., Dong, J., Lin, Z., 2014. miRNA-146 negatively regulates the production of pro-inflammatory cytokines via NF-κB signalling in human gingival fibroblasts. J. Inflamm. 11, 38. https://doi.org/10.1186/s12950-014-0038-z

Role of Nrp1 in Foxp3+Treg-dependent suppression mechanism

Mauricio Campos-Mora[1,2], Karina Alejandra Oyarce[2], Pamina Contreras-Kallens[2], Dario Vergara[2], Oscar Cerda[1], Karina Pino-Lagos[2]

[1]Universidad de Chile, Santiago, Chile
[2]University of the Andes, Chile, Las Condes, Chile
mcamposm@ug.uchile.cl; karina.oyarce@gmail.com; pamina.contreras@ug.uchile.cl; dario.vergara.slz@gmail.com; oscarcerda@uchile.cl; kpino@uandes.cl

Keywords: Exosomes, Transplantation, tolerance, rejection, Tregs, NRP1

Transplant rejection is a persistent clinical problem, in which the life-long use of immunosuppressive drugs is detrimental to the patient. Regulatory T cells (Tregs) are a key cell population in the maintenance of immune tolerance, and for instance, an interest target for cell therapy. This cell population presents distinctive markers, such as Foxp3, Eos, CD25 and membrane co-receptor Neuropilin-1 (Nrp1). Nrp1 has been associated with immune tolerance in several studies, although its function in Tregs is poorly understood. Tregs exert immune modulation through several mechanisms, such as cytokine production, target cell cytolysis, metabolic disruption and -recently described- exosome production, in order to inhibit immune responses and preserve homeostasis. Compared to other immune cells, Tregs secrete high amounts of exosomes with immunosuppressive capacity; however, the mechanisms of function of these Treg-derived exosomes in target cells are not yet fully known. Since Nrp1 is capable to interact with different target cell membrane proteins and reports had described its presence on murine and human exosomes, here we investigated the participation of Nrp1 in the immune function of Tregs and Treg-derived exosomes in homeostasis and allograft rejection.

Lymphocytes from blood and lymphoid organs were obtained from Treg-restricted-Nrp1-deficient animals (offspring of Foxp3/YFPCre bred with Nrp1-floxed, including heterozygous and WT mice as controls), and Tregs markers were analyzed by flow cytometry. Our results indicate that there is no difference in Eos expression on Nrp1-deficient Tregs, albeit modest differences in the expression of CD25, Nrp1 and Eos on conventional T CD4+ cells and T CD8+ cells. Consistent with our previous data, conventional CD4+ Nrp1- T cells increase Nrp1 expression when in vitro co-cultured with Nrp1+ Treg cells, which seems to be dependent on Nrp1 expression on Tregs since its absence did not favor the gain of Nrp1 or Eos by conventional CD4+ T cells Using in vitro suppressive assay, we observed that Nrp1-deficient Tregs exert diminished suppressive function, which correlates with their inability to tolerate a skin transplant

in vivo, which is associated with an increment in the frequencies of IFNγ+ and IL-17+ effector T cells. According with these observations, we also found that Nrp1-deficient Tregs express higher levels of IFNγ during allograft rejection, in comparison with their littermate controls. Regarding Tregs-derived exosomes, we could detect the presence of Nrp1 in the membrane of the exosomes, but the deletion of Nrp1 on Tregs did not affect exosome production. Finally, we demonstrate that Nrp1+ exosomes modulate in vitro activation of conventional CD4+ T cells, as seen by the inhibition of IFNγ mRNA production. Taking all together, our data suggest that Nrp1 participates in modulating the function of effector CD4+ T cells, and that Nrp1+ exosomes could be involved in this suppressive mechanism.

Evaluation of the epstein-barr virus (EBV)-specific T lymphocyte immune response in HIV+ patients in the different clinical stages of the disease and in patients with HIV-associated lymphoma

Diana Marcela Hernandez, Sandra Quijano

Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, Colombia
dianah939@gmail.com; squijano@javeriana.edu.co

Keywords: immune response, Epstein-Barr virus, human immunodeficiency virus, TCR-Vb repertoire, multifunctionality T

HIV infection (Human Immunodeficiency Virus) causes an immunodeficiency condition that contributes to the significant alteration in the immune surveillance against infectious agents favoring the development of tumors associated with oncogenic and persistent viruses, as is the case of opportunistic infection by the Epstein Barr virus (EBV) [1, 2].

EBV is a ubiquitous human herpesvirus gamma [3] that infects more than 95% of the world population, maintaining a lifelong asymptomatic infection due to the interaction of EBV with memory B lymphocytes, resulting in an infection latent in these cells in healthy adults [4, 5].The virus is etiologically related to a wide range of human tumors including B lymphomas, because it expresses a differential profile of viral oncogenic proteins in different latency phases [6] that are present in tumor cells of different tissues [7, 8].These latent oncogenic proteins contribute to tumor pathogenesis because they activate different signaling pathways that alter the cell cycle, apoptosis, cell differentiation and influence different mechanisms of migration and survival in tumor cells [9, 10].

Patients HIV+ have a tendency to develop aggressive non-Hodgkin's B (NHL-B) lymphomas, a type of cancer related to HIV-associated death. It has been described that patients HIV+ have a 60–200-fold greater risk of developing NHL-B and 8–10-fold Hodgkin's lymphomas compared to uninfected individuals [11] and these tumors are associated in a high percentage of cases with active infection with EBV, being detected for example in 60% of cases in Burkitt's lymphoma (BL) and in 100% in primary lymphomas of the central nervous system (PCNSL) [12].

Among the risk factors involved in the evolution to lymphomas in patients HIV+ [10] are the high levels of HIV plasmaviremia (> 100,000 copies RNA/ml), high viral load of oncogenic viruses such as EBV, low CD4+ T cell counts [12], the advanced state of

infection [11] the loss of multifunctionality and diversity of the TCR-VB repertoire [13, 14] and the exhaustion phenomenon in memory T lymphocytes [3, 13]. Taking into account these antecedents, this work has been directed to answer the following research question: Are there changes in the TCR-VB repertoire, in the monocyte and multifunctional memory T-cell cytokine profile and cytokines specific to EBV in patients HIV+ in the different clinical stages of the disease?.

The general objective of this work was to evaluate the frequency, diversity and functionality of the $CD4^+$ and $CD8^+$ T lymphocyte response specific to Epstein-Barr Virus (EBV) in HIV^+ patients at different clinical stages of the disease and in patients with B lymphomas associated with HIV. For this study, peripheral blood (PB) from 62 patients HIV+ from San Ignacio University Hospital was used in different clinical stages (16 patients in Stage 1, 20 in Stage 2, 20 in Stage 3 and 6 with non-Hodgkin B lymphoma) and 27 healthy controls. The samples were stimulated with a EBV lysate by evaluation of the presence of $CD4^+$ and $CD8^+$ memory compartments (CD45RA/CCR7), the frequency of functional T producing TNF-a, IFN-g and IL-2 (intracellular), soluble cytokines (IL-4, IL-6, IL-10, TNF-a, and IFN-g) and specific TCR-VB repertoire (24 families) by flow cytometry. As a functional control, PB samples were stimulated with PMA-Ionomycin.

In the present study we observed that Compared with healthy controls, the patients HIV+ in advanced stages of the disease (stage 3 and B lymphoma) had a significant decrease in all subpopulations of CD4+ T lymphocytes (virgins, central memory, effector memory and effectors) and in patients with lymphoma an evident loss of CD8+ T lymphocytes of effectors with predominance of the effector memory compartment compared to healthy individuals ($p < 0.05$ respectively). Importantly, in all patients HIV+ there was a decrease in EBV-specific CD4+ T cells producing IL-2 and was found and patients in advanced stages of the disease (stage 3 and lymphoma) had lower CD4+ and CD8+ T lymphocyte counts of TNF-a+ and IFN-g+ compared to healthy controls ($p < 0.05$ respectively); together with absence of multifunctional CD4+ and CD8+ T lymphocytes (simultaneous producers of TNF-a+/IFN-g+/IL-2+). The analysis of soluble cytokines also showed decreased concentrations of TNF-a and IL-2 and a very low or absent IFN-g producción production in all patients HIV+.

Finally, it was observed that patients HIV+ have a less diverse TCR-VB repertoire with less representation of T lymphocyte families that are more frequent in healthy individuals. Furthermore, in response to EBV, patients HIV+ showed a significant decrease in the VB2, VB4, VB7.1, VB9, VB13.6, VB14, VB17 and VB22 families in the T lymphocytes. CD4+ and families VB14 and VB17 in CD8+ T lymphocytes. Which play a key role in the protection against EBV in healthy individuals. These results show a lower immunological surveillance against EBV, which could favor its reactivation in

B lymphocyte populations and its ability to transform these cells into tumor cells. In addition, these findings together suggest that the loss of immunosurveillance against EBV may increase the risk of clinical evolution to B-cell lymphomas in these patients.

REFERENCES

1. Maartens, G., C. Celum, and S.R. Lewin, HIV infection: epidemiology, pathogenesis, treatment, and prevention. Lancet, 2014. 384(9939): p. 258-71.
2. Carbone, A., et al., Epstein-Barr virus associated lymphomas in people with HIV. Curr Opin HIV AIDS, 2017. 12(1): p. 39-46.
3. Ressing, M.E., et al., Immune Evasion by Epstein-Barr Virus. Curr Top Microbiol Immunol, 2015. 391: p. 355-81.
4. Taylor, G.S., et al., The immunology of Epstein-Barr virus-induced disease. Annu Rev Immunol, 2015. 33: p. 787-821.
5. Young, L.S., L.F. Yap, and P.G. Murray, Epstein-Barr virus: more than 50 years old and still providing surprises. Nat Rev Cancer, 2016. 16(12): p. 789-802.
6. Dolcetti, R., et al., A lymphomagenic role for HIV beyond immune suppression? Blood, 2016. 127(11): p. 1403-9.
7. Castillo, J.J., et al., Epstein-barr virus-positive diffuse large B-cell lymphoma of the elderly: what we know so far. Oncologist, 2011. 16(1): p. 87-96.
8. Geng, L. and X. Wang, Epstein-Barr Virus-associated lymphoproliferative disorders: experimental and clinical developments. Int J Clin Exp Med, 2015. 8(9): p. 14656-71.
9. Gruhne, B., et al., EBV and genomic instability--a new look at the role of the virus in the pathogenesis of Burkitt's lymphoma. Semin Cancer Biol, 2009. 19(6): p. 394-400.
10. Castillo, J.J., et al., Viral lymphomagenesis: from pathophysiology to the rationale for novel therapies. Br J Haematol, 2014. 165(3): p. 300-15.
11. Gloghini, A., R. Dolcetti, and A. Carbone, Lymphomas occurring specifically in HIV-infected patients: from pathogenesis to pathology. Semin Cancer Biol, 2013. 23(6): p. 457-67.
12. Petrara, M.R., et al., Epstein-Barr virus-driven lymphomagenesis in the context of human immunodeficiency virus type 1 infection. Front Microbiol, 2013. 4: p. 311.
13. Cardenas, D., et al., Epstein-Barr virus-specific CD8(+) T lymphocytes from diffuse large B cell lymphoma patients are functionally impaired. Clin Exp Immunol, 2015. 182(2): p. 173-83.
14. González-Serna, A., et al., CD8 TCR β chain repertoire expansions and deletions are related with immunologic markers in HIV-1-infected patients during treatment interruption. Journal of Clinical Virology, 2013. 58(4): p. 703-709.

Lipopolysaccharide induces CD38 expression on effector and memory CD4+ and CD8+ T cells, and the presence of this protein enhances its proliferation and effector responses

Wendolaine Santiago Cruz[1], Jocelyn Carolina Pérez Lara[1], Enrique Espinosa[2], Hector Romero[3], Juan Carlos Rodriguez Alba[1]

[1]Universidad Veracruzana, Xalapa, Mexico
[2]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
[3]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
wendii.santiago@gmail.com; Jocelyn1908@hotmail.com; hector.enrique.espinosa@gmail.com; hromero@cinvestav.mx; JCRA19@yahoo.com

Keywords: LPS, effector T cells, memory T cells, CD38, T cells proliferation

CD38 is a 42 kDa transmembrane glycoprotein widely expressed by hematopoietic cells. This protein possesses both receptor and ectoenzyme activities. The CD38 receptor activity has been related with activation, proliferation and cytokines production by lymphocytes after an anti-CD38 mAb stimulus. Several reports shown that lipopolysaccharide (LPS) from gram-negative bacteria is able to induce polyclonal activation of B cells and increases the CD38 levels, but there are no reports concerning the expression and function of this protein on neither CD4+ nor CD8+ T cells subpopulations. In this work, we evaluated expression of CD38 on splenic CD4 or CD8 T cells expressing CD44low, CD62Lhigh (naïve), CD44high, CD62Llow (memory), CD44high, CD62Llow (effector) from C57BL/6 mice stimulated with LPS in "in vitro" assays. Additionally, we evaluate the response of these same subsets to LPS in the absence of CD38, using total splenocytes from B6.CD38-/- mice. Our results shown that LPS induces an increase in CD38 expression by CD8 memory and effector T cells in C57BL/6 mice. These same subsets do not proliferate at the same level in the CD38-deficient mice, and the absence of CD38 reduces the activation mediated by LPS in memory and effector from CD4+ and CD8+ lymphocytes. The determination of the IFN-gamma and IL-6 production will further help to elucidate the role of CD38 in the cytokines modulation. Fondo CONACyT Fronteras de la Ciencia 214 (convocatoria 2015).

Does functional up-regulation of KCa3.1 channels influences the proliferative potential of leukemic cells?

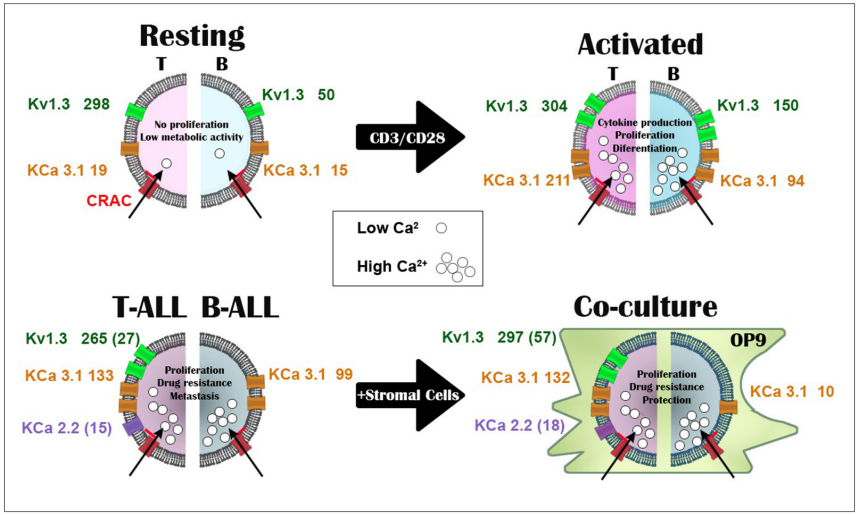
Salvador Valle Reyes[1], Igor Pottosin[1], Rosana Pelayo[2], JC Balandrán[2], Miguel Ángel Olivas[1], Laura Hadit Cruz Aguirre[1], Oxana Dobrovinskaya[1]

[1]University of Colima, Colima, Mexico
[2]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
juanchavador@hotmail.com; pottosin@ucol.mx; rosana.pelayo@imss.gob.mx; jcbalandran@gmail.com; miguel.a.olivas@gmail.com; laura_hadit_13@hotmail.com; oxana@ucol.mx

Keywords: Potassium Channels, CRAC channel, T-ALL, Co-culture model, B- ALL, Pathc Clamp

Antigen activation of naïve and memory lymphocytes leading to proliferation, clone expansion and differentiation into effector cells represents one of the most-studied events in T- and B-cell biology. Approximately 75% of all genes upregulated during lymphocyte activation are shown to be dependent on calcium (Ca2+) rise. Principal Ca2+ influx in lymphocytes involves a coordinated interplay of several ion channels. To begin with, Ca2+ release-activated Ca2+ channel, known as CRAC, was shown to be a core element for the Ca2+ influx during T cell activation (1, 3). On the other hand, there are potassium (K+) channels that control the plasma membrane potential and play a key role in regulating of the Ca2+ influx. Particularly, in T-lymphocytes, voltage-dependent K+ channels Kv1.3 and Ca2+-activated K+ channels KCa3.1 are involved in this regulation. It is widely accepted that specifically shaped Ca2+ signals (Ca2+ signature) define such processes as maturation, activation, and differentiation of healthy lymphocytes (5, 7).

Acute lymphoblastic leukemia (ALL) is a malignant disorder resulting from the clonal proliferation of lymphoid precursors with arrested maturation. Importantly, the majority of signaling pathways up-regulated in leukemic cells are also Ca2+-dependent. Although ion channels´ patterns in healthy mature lymphocytes, both resting and activated, are studied extensively, there are currently no data on immature progenitors and/or leukemic cells, with the exception of better studied Jurkat cell line (2, 1). It is worth noting here that, although ion channels expression may be characterized at the protein or mRNA levels, these approaches do not provide direct information on the ion channels functional activity, which can be evaluated only by electrophysiological measurements on single cells (4, 7).



In the present work, we applied patch-clamp technique to study functional expression of Kv and KCa currents in leukemic T (human Jurkat, CEM and MOLT-3) and B (human RS4:11 and REH cell) lineages. Taking into account the accumulating evidence showing the crucial role of bone marrow niche for maintenance and drug resistance of leukemic cells, some experiments were carried out with leukemic cells co-cultured with stromal (OP9) cells. The measurements of K+ currents were combined with a specific pharmacology, providing their identification.

Similar to primary CD4+ cells, the voltage-dependent K+ current in leukemic T cells was solely represented by Kv1.3 channel. KCa currents were dominated by KCa3.1 channels in healthy T cells as well as leukemic T cell lines CEM and MOLT-3. The level of KCa expression in activated T lymphocytes and leukemic T cells was similar and 10-fold higher than in resting T cells. In contrast, Jurkat cells displayed a very unusual profile, with a 10- fold lower Kv (Kv1.3) and KCa currents density as compared to CEM, MOLT and activated T cells from the peripheric blood of healthy donors. Instead of KCa3.1 channels, KCa current in Jurkat was dominated by small conductance KCa2.2 channels, which are normally expressed in brain. The level of functional KCa expression in Jurkat was comparable to resting T cells. Bearing in mind a high proliferative potential of Jurkat cells, low expression of Kv and KCa channels looks counterintuitive, and suggests alternative regulatory mechanisms of Ca2+ fluxes in some subsets of T leukemic cells. Different types of K+ channels may be also involved in this regulation, for example TRESK channel, belonging to the K2P family (6), as was demonstrated by our group in previous studies.

In B cell models RS4:11 and REH we were unable to detect Kv current, whereas KCa current was dominated by KCa3.1.

The functional expression of Kv and KCa channel was also undertaken in leukemic T- and B-cells co-cultured with stromal cells, imitating leukemic niches in bone marrow. Co-culturing caused a modest increase of Kv1.3 and KCa currents in Jurkat, CEM and MOLT cell lines. In RS4:11 the KCa3.1 current was decreased significantly, whereas in REH there were no significant changes. Thus, B and T leukemic cell lines differentially interact with supporting cells within an in vitro simulated niche.

We speculate that more commonly (except an exotic case of Jurkat cell line), KCa3.1 channels, which are up-regulated upon the activation of healthy T cells, also play a key role in control of cellular functions and proliferation of leukemic B- and, especially, T-cells. Consequently, available non-toxic specific blockers, developed against KCa3.1 channels, maybe proposed for the T-ALL treatment. Thus, additional studies on primary cells cultures, derived from leukemic patients, are needed to strengthen this suggestion.

REFERENCES

1. Abraham RT, and Weiss A (2004). Jurkat T cells and development of the T-cell receptor signalling paradigm. Nat.Rev. Immunol. 4, 301-308. doi:10.1038/nri1330.
2. Dobrovinskaya O, Delgado-Enciso I, Quintero-Castro LJ, Best-Aguillera C, Rojas-Sotero RM, and Pottosin I (2015). Placing ion channels into signaling network of T cells: from maturing thymocytes to healthy T lymphocytes and leukemic T lymphoblasts. Biomed. Res. Int. 2015, 750203, doi: 10.1155/2015/750203.
3. Feske S, Skolnik EY, and Prakriya M (2012). Ion channels and transporters in lymphocyte function and immunity. Nat. Rev. Immunol. 12, 235-247. doi: 10.1038/nri3233.
4. Manabe A, Coustan-Smith E, Behm FG, Raimondi SC, Campana D (1992) Bone marrow-derived stromal cells prevent apoptotic cell death in B-lineage acute lymphoblastic leukemia. Blood; 79: 2370–2377.
5. Panyi G (2005). Biophysical and pharmacological aspects of K channels in T lymphocytes. Eur. Biophys. J. 34, 515-529. doi: 10.1007/s00249-005-0499-3.
6. Pottosin I, Bonales-Alatorre E, Valencia-Cruz G, Mendoza-Magaña ML, Dobrovinskaya O (2008) TRESK-like potassium channels in leukemic T cells. Pflugers Arch 456(6): 1037-1048.
7. Szabo I, Trentin L, Trimarco V, Semenzato G, and Leanza L (2015). Biophysical characterization and expression analysis of Kv1.3 potassium channel in primary human leukemic B cells. Cell. Physiol. Biochem. 37, 965-978. doi: 10.1159/000430223.

Cannabidiol suppresses human leukemic T cells via breakdown of the mitochondrial function: the role of intracellular Ca2+

Miguel Olivas-Aguirre, Miguel Flores, Liliana Torres, Juan Salvador Valle, Liliana Liñán, Igor Pottosin, Oxana Dobrovinskaya

University of Colima, Colima, Mexico
miguel.a.olivas@gmail.com; flores_miguel@ucol.mx; lilianatorres4667@gmail.com; Juan_valle@ucol.mx; lilian-lr@hotmail.com; pottosin@ucol.mx; Oxana@ucol.mx

Keywords: Calcium, Cannabidiol, Cell Death, Leukemia, Mitochondria, cbd, T-ALL

Acute Lymphoblastic Leukemia (ALL) is a malignant hematological disorder where lymphoid cells exhibit abnormal characteristics that involves poor maturation, high proliferation, death resistance and drug evasion. The incidence of T cell-lineage ALL (T-ALL) has a bimodal distribution with a peak around the first 4 years of life of the patients and a less robust peak in the late stages of life (1). Upon diagnostics, traditional therapy includes the use of integrative regimens based on the use of radiotherapy, chemotherapeutic compounds and allogeneic stem cell transplantation, where up to 80% of the patients respond favorably. However, most of them suffer multiple relapses and despite of the opportune diagnostic and a wide variety of chemotherapeutical agents available, only about 30% of patients will obtain long term remission (2). This partial success has encouraged the scientific community to look for novel strategies for T ALL treatment.

Cannabidiol (CBD) is a major non-psychotropic derivative from marijuana (Cannabis spp.). It has been postulated as a potential anticancerigenous compound as it drives cancer cells (i.e. prostate, breast, glia) to cell death by mechanisms, which are not well understood yet (3). In this study we analyzed the effects of CBD on the intracellular Ca2+ dynamics, viability and migration capacity of human leukemic cells. Lymphoblastic cell lines Jurkat, MOLT-3 and CEM derived from T-ALL patients in relapse were used as models.

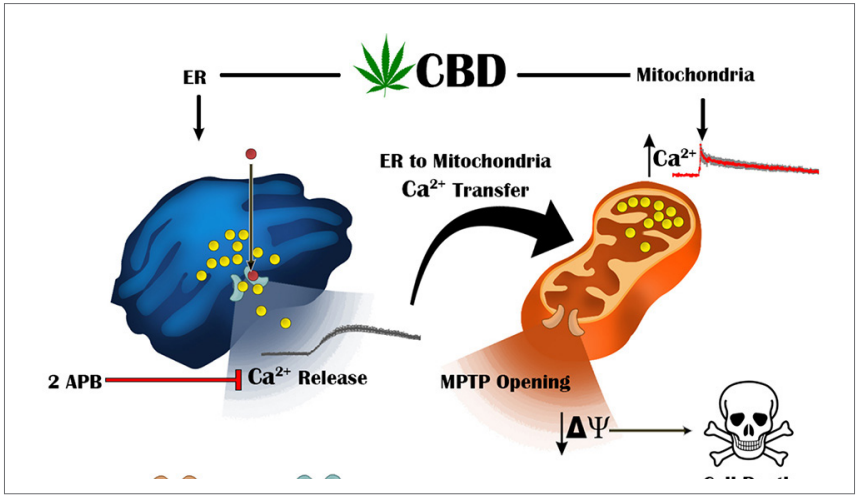
Cytosolic Ca2+ was analyzed in cell populations, loaded with a ratiometric fluorescent indicator FURA-2 (Molecular Probes). To monitor cytosolic Ca2+ changes, samples were excited at 340/380 nm and emission was collected at 510nm. In all cell models CBD (Cayman Chemical) induced a significant (dose-dependent, about 3-fold of the resting level with 30 mM CBD) increase of cytosolic Ca2+, peaked at 1.5 min. This increase was neither dependent on extracellular Ca2+ nor it was mediated by known

cannabinoid receptors (CB1, CB2, GPR55). CBD-induced cytosolic transient was abolished by inositol-3-phosphate receptor channels inhibition or by a depletion of endoplasmic reticulum (ER), implying the latter Ca2+ store as a principle source of cytosolic Ca2+ rise. Yet, the inhibition of CBD-induced Ca2+ release from the ER did not revert the strong suppression of the metabolic activity caused by CBD in all T-ALL lines, as it was assessed by resazurin-based assay (Tox 8- Sigma-Aldrich).

Thus, we were looking for additional intracellular targets for CBD, which may affect T-ALL function and survival. Strikingly, we observed that CBD rapidly (in few minutes) collapsed mitochondrial membrane potential (Ψm), monitored as a loss of specific fluorescence of a cationic TMRE dye (MitoTrackerTM Green FM was used for specific mitochondria labeling), whose accumulation in the mitochondria matrix requires matrix-negative electric potential difference across the inner mitochondrial membrane. Even more surprisingly was the fact that CBD displayed even higher potency as compared to a classical uncoupler, FCCP, commonly used to dissipate the Ψm across mitochondrial inner membrane. Large negative Ψm across mitochondrial inner membrane is a mandatory pre-requisite for the ATP synthesis by means of oxidative phosphorylation. It is not surprising that running short of the main cell energy currency, ATP, CBD-treated T-ALL cells have shown a largely reduced mobility and metabolism (survival), as has been demonstrated in respective assays.

Such a rapid collapse of Ψm, as observed upon the CBD application, indicates a stable opening of so called mitochondrial transition pore (MTP). Formation of the MTP requires a very significant free Ca2+ increase in the mitochondrial matrix, from the resting level of 0.1 mM to ~10 mM (4). Measuring mitochondrial Ca2+ remains a rather challenging task. We found out that a conventional mitoCa2+ fluorophore, Rhod2, is very promiscuous and localizes also in off target intracellular structures. Thus, we opted for a new class of genetically encoded Ca2+ indicators, named as calcium-measuring organelle-entrapped protein indicators (CEPIA), in particular CEPIAmt, which rather specifically targets mitochondria. Of those CEPIAmt3 was of particular choice, due to its high Ca2+ concentration operation range (apparent KD = 11 mM) (5).

We were able to successfully functionally express CEPIAmt3 in Jurkat cells. Samples were excited at 488 nm and fluorescence was collected at 510 nm. Application of CBD provoked a very fast (<1 min) and huge (>10 mM) Ca2+ increase in the mitochondrial matrix, sufficient for a complete activation of the MTP. Concurrent measurements of CBD-induced Ca2+ release from the ER to cytosol and Ca2+ rise in mitochondrial matrix indicated that mitochondrial Ca2+ overload is potentiated by Ca2+ release from the ER, yet the inhibition of the latter did not abolished the CBD-induced mitochondrial Ca2+ transient. CBD-induced mitochondrial Ca2+ rise was increased and stabilized in the presence of CsA, which potently inhibits MTP. Thus, MTP, which is



responsible for the collapse of ¨m, is activated by mitochondrial Ca2+ overload, but at the same time serves to discharge excessive Ca2+ back to the cytosol. Opening of MTP is associated with mitochondrial swelling, breakdown, release of apoptotic factors (cytochrome c), and induction of the cell death (6). CBD induces a parallel release of Ca2+ from ER and increase of mitochondrial Ca2+; yet the two processes may also interact one with another, so that local ER to mitochondria Ca2+ transfer may take place, increasing the mitochondrial Ca2+ overload. Our work represents a starting point for the understanding of alternative mechanism for the CBD signaling, which is not mediated by cannabinoid receptors. Central for this mechanism is the CBD-induced mitochondrial Ca2+ overload, leading to the cell death. It opens the perspective for CBD usage in the antileukemic therapy.

Figure 1 represents a graphical abstract of the present work.

REFERENCES

1. Hossain JM, Xie L, McCahan SM. Characterization of Pediatric Acute Lymphoblastic Leukemia Survival Patterns by Age at Diagnosis. J.Cancer Epidemiol (2014) doi:10.1155/2014/865979
2. Siegel RL, Miller DK, Jemal A. Cancer statistics, 2016. Ca Cancer J Clin. (2016) doi:10.3322/caac.21332
3. Massi P, Solinas M, Cinquina V, Parolaro D. Cannabidiol as a potential anticancer drug. JClin Pharmacol (2013). doi: 10.1111/j.1365-2125.2012.04298
4. Chalmers S, Nichonlls DG. The relationship between Free and Total Calcium Concentrations in the Matrix of Liver and Brain Mitochondria J Biol Chem (2003) doi: 10.1074/jbc.M212661200

5. Suzuki J, Kanemaru K, Ishii K, Ohkura M, Okubo Y, Iino M. Imaging intraorganellar Ca2+ at subcellular resolution using CEPIA. Nat Commun (2014) doi: 10.1038/ncomms5153
6. Ott M, Robertson JD, Gogvadze V. Zhivotovsky B, Orrenius S. Cytochrome C release from mitochondria procees by a two-step process. PNAS (2002) doi: 10.1073/pnas.241655498

Estrogen receptors modulators demonstrate antileukemic properties in human acute lymphoblastic T-cell leukemia cell line Jurkat

Liliana del Rocío Torres-López[1], Iván Delgado-Enciso[1], Paola Maycotte-González[2], Andrómeda Liñán-Rico[1], Luis Donis-Maturano[3], Salvador Valle-Reyes[1], Liliana Liñán-Rico[1], Miguel Ángel Olivas-Aguirre[1], Oxana Dobrovinskaya[1]

[1]University of Colima, Colima, Mexico
[2]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
[3]Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico
lilianatorres4667@gmail.com; ivancoliman@hotmail.com; bisbenzimida@gmail.com; andromeda.lr@gmail.com; ludoma5@yahoo.com.mx; juanchavador@hotmail.com; lilian-lr@hotmail.com; miguel.a.olivas@gmail.com; oxana@ucol.mx

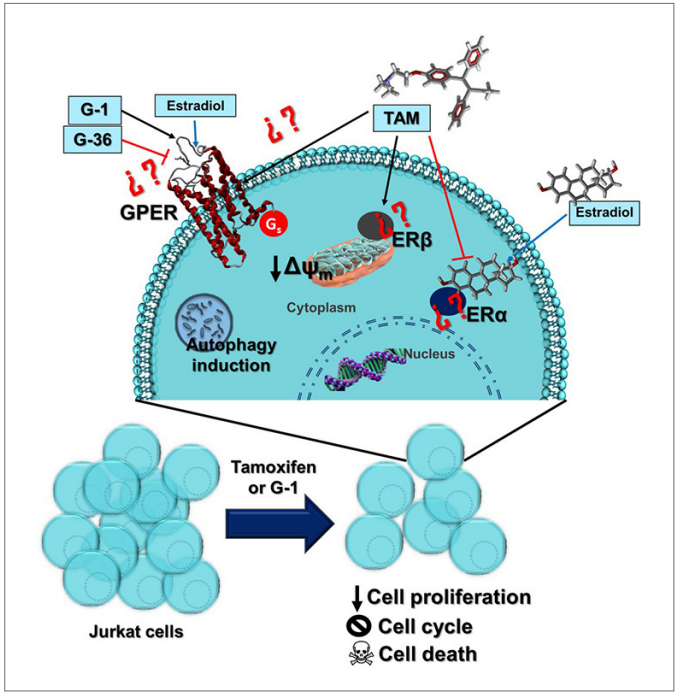
Keywords: Leukemia, Tamoxifen, Acute Lymphoblastic Leukemia, estrogen receptors, Jurkat

Estrogens demonstrate biological activity in numerous organ systems, including the immune system and exert their effects through estrogen receptors (ERs) of two types: intracellular ERα and ERβ that activate transcriptional factors and membrane G protein-coupled ERs GPER, also known as GPR30. The latter are capable to mediate fast activation of cytosolic signaling pathways, influencing transcriptional events in response to estrogens (5). ERs were shown to be expressed in polymorphonuclear and mononuclear leucocytes isolated from the peripheral blood of both men and women (8).

To date, several ERs modulators have been described. Tamoxifen (TAM), widely used in chemotherapy of breast cancer, is considered as an ERα antagonist and GPER agonist (9). Interestingly, TAM was shown to present cytotoxicity in various cell types, including those that do not express intracellular ERs. Although TAM was included in protocols for treatment of melanoma (6) and pediatric rhabdoid tumors (2), the mechanism of its cytotoxicity in case of these cancers remain elusive. More recently, highly specific GPER ligands have been synthesized: G-1 and G-36 molecules that have agonist and antagonist properties, respectively (1,3,4). Cytotoxic and antiproliferative effect of G-1 was reported for lung, breast and ovarian cancer. Then G-1 was proposed as a novel promising anticancer drug.

Present study was designed to characterize the biological effects and therapeutic antileucemic potential of three estrogen receptor modulators mentioned above, in T cell Acute Lymphoblastic Leukemia (T-ALL). As a model, CD4+-positive leukemic

Jurkat cell line was used, which was reported as ER-α-negative (7). The cells were treated with different concentrations of TAM, G-1 and G-36. At different times after drug application, biological drug effects were evaluated by means of diverse functional assays using spectrofluorimetry, cytometry and confocal microscopy. The number of viable cells was evaluated by resazurin-based metabolic assay and cell count. Cell proliferation was estimated by monitoring of fluorescence intensity of intracellular cell tracer CFSE (Carboxyfluorescein Diacetate Succinimidyl Ester), halving by each cell division. Fluorescent DNA-binding dye Propidium Iodide (PI) was used to evaluate the cell cycle progression basing on the DNA-content relation. Cationic fluorescent dye Rhodamine-123 was employed to evaluate the changes in mitochondrial membrane potential (Δψm). To determine the type of cell death, Alexa Fluor 488-conjugated Annexin-V (phosphatidyl serine detection characteristic for apoptosis process) and PI (necrosis process) were used. Autophagy was evaluated through autophagosome detection with fluorescent dye Monodancyl cadaverine (MDC). Alternatively, autophagic flux was estimated in Jurkat cells stably expressing mCherry-GFP-LC3 (a gift from Dr. Andrew Thorburn' lab, Denver, CO, USA) as an autofagic reporter. Additionally



LC3-I/II detection through western blot was undertaken. Finally, changes in genic expression was evaluated by ARN microarray and Real-Time PCR.

The modulator TAM reduced the cell viability and cell proliferation at concentration > 7.5 μM. This cytotoxic effect was not prevented by GPER antagonist G-36 suggesting that cytotoxic and antiproliferative effect of TAM is not mediated by binding to the estrogen receptors. TAM also produced inhibition of the cell cycle progression and accumulation of cells in G1 phase at concentrations within 5–10 μM range (24 hours of treatment). The level of autophagy increased gradually, in concentration-dependent manner and the co-treatment with an autophagy blocker, chloroquine, reduced the cell viability. TAM (7.5μM) also targeted mitochondria and caused $\Delta\psi m$ loss, which started 4 hours after the initiation of treatment. At concentrations > 10 μM, TAM caused both necrosis and apoptosis. Finally, our findings were corroborated by ARN microarray analysis. In particular, the overexpression of genes related to apoptosis (BCLAF1, CFLAR, CARD8, TRADD, DIDO1) and autophagic processes (ATG5), as well as the downregulation of genes related to cell proliferation (IL9, IL4) and metabolism (COX7A2L, CYP1B1) were revealed. Highly specific GPER agonist G-1 was more efficient than TAM: it reduced cell viability, induced apoptosis and $\Delta\psi m$ at concentrations as low as 0.75 μM after 24 hours of treatment. Collectively, the data presented here shed light on the cellular mechanisms of the TAM action on leukemic cells and provide plausible explanations for its therapeutic potential.

REFERENCES

(1) Bologa CG, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, et al. Virtual and biomolecular screening converge on a selective agonist for GPR30. Nature Chemical Biology (2006) 2:4. doi:10.1038/nchembio775.

(2) Cimica V, Smith ME, Zhang Z, Mathur D, Mani S and Kalpana GV. Potent inhibition of rhabdoid tumor cells by combination of flavopiridol and 4OH-tamoxifen. BMC Cancer (2010) 10:634.

(3) Dennis MK, Burai R, Ramesh C, Petrie WK, Alcon SN, Nayak TP, et al. In vivo effects of a GPR30 antagonist. Nat Chem Biol (2009) 5:6. doi:10.1038/nchembio.168.

(4) Dennis MK, Field AS, Burai R., Ramesh C., Petrie WK, Bologa CG, et al. Identification of a GPER/GPR30 antagonist with improved estrogen receptor counterselectivity. J Steroid Biochem Mol Biol (2011) 127:3-5. doi: 10.1016/j.jsbmb.2011.07.002.

(5) Filardo EJ, Quinn JA, Frackelton AR Jr and Bland KI (2002). Estrogen action via the G Protein-Coupled Receptor, GPR30: Stimulation of Adenylyl Cyclase and cAMP-mediated attenuation of the Epidermal Growth Factor Receptor-to-MAPK signaling axis. Molecular Endocrinology 16:1.

(6) McClay EF and McClay ME. Tamoxifen: Is it useful in the treatment of patients with metastatic melanoma? Journal of Clinical Oncology (1994) 12:3.

(7) Nagahara Y, Shiina I, Nakata K, Sasaki A, Miyamoto T and Ikekita M. Induction of mitochondria-involved apoptosis in estrogen receptor-negative cells by a novel tamoxifen derivative, ridaifen-B. Cancer Sci vol (2007) 99:3. doi: 10.1111/j.1349-7006.2007.00709.x.

(8) Pierdominici M, Maselli A, Colasanti T, Giammarioli AM, Delunardo F, Vacirca D, et al. Estrogen receptor profiles in human peripheral blood lymphocytes. Immunology Letters (2010) 132:1-2. doi: 10.1016/j.imlet.2010.06.003.

(9) Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA and Hathaway HJ. Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. Annu. Rev. Physiol (2008) 70. doi: 10.1146/annurev.physiol.70.113006.100518.

Influence of CRACC on CD8+ T cell function

Arturo Gutiérrez Guerrero[1], André Veillete[2], Mario Ernesto Cruz Muñoz[1]

[1]Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[2]Faculté de médecine, Université de Montréal, Montreal, Canada
arturo.gutierrezge@uaem.edu.mx; andre.veillette@ircm.qc.ca; mario.cruz@uaem.mx

Keywords: CD8+ T cells, T cell biology, degranulation, Cell cytotoxicity, CRACC

Introduction: CRACC (CD2-like receptor activating cytotoxic cells, also called CD319, CS1 or SLAMF7) is a molecule expressed on cells of the immune system. CRACC is expressed by natural killer (NK) cells, B cells, CD4+ and CD8+ T cells, dendritic cells, plasma cells, and macrophages. This molecule is a member of the signaling lymphocytic activation molecule (SLAM) family. Similar to other members of SLAM family, CRACC is hemophilic receptors and thus functions as a self-ligand. The SLAM family receptors display diverse functions including roles in adhesion between hematopoietic cells, T cell cytokine production, co-stimulation, NK cell and CD8+ T cell-mediated cytotoxicity, and most recently described, phagocytosis. Through immunoreceptor tyrosine-based switch motifs (ITSMs) located within their cytoplasmic domain, SLAM family receptors associate with the SLAM-associated protein (SAP) family of adaptor proteins. The members of the SAP family include SAP (SLAM-asociated protein) and EAT-2 (Ewing´s sarcoma-activated transcript 2). All SLAM family receptors bind SAP and EAT-2, except for CRACC, which only binds EAT-2. In mouse NK cells, CRACC/EAT-2 association promotes NK cell-mediated cytotoxicity, whereas in the absence of EAT-2, CRACC favors inhibition of NK cell-mediated cytotoxicity. Both, activation and inhibition are dependent on different tyrosine residues in the cytoplasmic domain of CRACC. In contrast, in human NK cells, CRACC seems to promote only NK cell inhibition, although an inhibitory role of CRACC has not been formerly studied. Finally, in macrophages CRACC promotes the phagocytosis of hematopoietic tumor cells by a mechanism independent of the SAP-related adaptors, suggesting that CRACC can regulate function of immune cells through various mechanisms. In mouse models, it has been suggested that CRACC engagement promotes cytotoxic lysis of target cells in response to viral antigens. Interestingly, CRACC is also express in human CD8+ T cells, although the specific role and mechanisms of action of CRACC in these cells remains unknown. Therefore, the aim of this study is to determine whether CRACC plays a specific role in human CD8+ T cells and the mechanism of action.

Materials and Methods: Peripheral blood mononuclear cells (PBMCs) were enriched by density centrifugation (Ficoll-Paque) and/or negative selection. First, we analyzed the expression of CRACC in various CD8+ T cell subsets. In addition, PBMCs were stimulated for different times with anti-CD3 and anti-CD28 monoclonal antibodies to mimic TCR-induced signaling, or with PMA/ionomycin to induce full cell activation. Following cell stimulation, CRACC expression was evaluated by flow cytometry at different time points. Likewise, we evaluated the impact of CRACC crosslinking on CD8+ T cell function such as cell adhesion, cell cytotoxicity, cytokine secretion and proliferation.

Results: T cell subsets from unstimulated PBMCs showed differential CRACC expression. Whereas naïve T cells lacked CRACC expression, memory and effectors T cells showed increased CRACC expression on the cell surface, suggesting a correlation between CRACC expression and T cell activation. Moreover, CRACC engagement in CD8+ T cells surface, affected different discrete steps of CD8+ T cell effectors functions such as cell adhesion, polarization and degranulation of cytotoxic granules. Our results suggest that CRACC play an important role in regulate CD8+ T cell function.

Genetic and molecular characterization of WAS patients in Mexican population

Jonathan Gilberto Cuellar Vega[1], Sara Elva Espinosa Padilla[2], Maria Edith Gonzáles Serrano[2], Esmeralda Nancy Jiménez Polvo[3], Edna Venegas[2], Ana Paola Macias Robles[4], Beatriz Adriana LLamas Guillén[3], Mario Ernesto Cruz Muñoz[1]

[1]Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[2]Instituto Nacional de Pediatria, Mexico City, Mexico
[3]Hospital del Niño y el Adolescente Morelense, Emiliano Zapata, Mexico
[4]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
jhoncu88@hotmail.com; saraelvaespino@gmail.com; megpetite@gmail.com; nancyjp_19@hotmail.com; e.venegasm@hotmail.com; paola_maro@hotmail.com; BEADRILUZ@hotmail.com; Mario.cruz@uaem.mx

Keywords: Cytotoxicity, Immunologic, Wiskott-Aldrich Syndrome, T cell subsets, inmunodeficiencias, degranulation

The Wiskott-Aldrich syndrome (WAS) is a primary immunodeficiency linked to the X chromosome, caused by mutations in the gene that encodes the protein WASp. This protein is expressed in non-erythrocyte hematopoietic cells and is a key regulator of actin cytoskeleton dynamics, playing an important role in essential processes such as development and activation of lymphocytes, chemotaxis, phagocytosis, cellular signaling, vesicular traffic, immune regulation, cellular mobility, cell-cell interactions, and cellular cytotoxicity. Due to its multiple functions, this protein is necessary for the correct development of innate and adaptive immune responses.

Mutations in the WAS gene may cause several clinical manifestations depending on the type and the site of the mutation. Three varieties of WAS have been described: classic WAS, XLT (X-linked thrombocytopenia), and neutropenia linked to the X chromosome. In classical WAS, the mutation in WASp causes the absence of the protein, therefore these are the most severe cases of the disease, with clinical manifestations including thrombocytopenia, eczema, increased susceptibility to infections, autoimmunity, and a major trend to develop lymphoma. In XLT, the mutation in WAS results in decreased WASp expression, with less severe symptoms characterized mainly by thrombocytopenia and sometimes the presence of milder eczemas. The neutropenia linked to the X chromosome is characterized by low neutrophil and monocyte numbers and an increase in the susceptibility to bacterial and fungal infections.

We have analyzed samples from 18 Mexican pediatric patients with clinical manifestations associated to WAS. Using flow cytometry analyses and molecular diagnostic tests, we have identified eleven mutations: six deletions that inhibit WASp expression

and four mutations that result in the expression of truncated WASp. Furthermore we analyzed the immunologic profile of each WAS patient and we correlated it with the type of mutation that it presents.

In order to determine the functional consequences of the mutations, we evaluated the cytotoxic response of NK cells, CD8+ T lymphocytes, and CD8+/CD57+ T cells. We quantified degranulation responses using flow cytometry based assays, measuring the surface expression of the CD107a marker on NK cells and CD8 T lymphocytes. Our results show that patients with truncated WASp expression also exhibit impaired cytotoxic function, both in NK and in cytotoxic CD8+ T lymphocytes, suggesting a role for WASp in the regulation of effector cell responses.

Application of a molecular algorithm for the identification of genetic causes associated with hemophagocytic lymphohystiocytosis

Mitzi Vanessa Antunez Barrera[1], Mario Ernesto Cruz Muñoz[1], Selma Cecilia Scheffler Mendoza[2], Sara Elva Espinosa Padilla[2]

[1]Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[2]Instituto Nacional de Pediatria, Mexico City, Mexico
alisonantunez@hotmail.com; mario.cruz@uaem.mx; drascheffler@hotmail.com; saraelvaespino@gmail.com

Keywords: degranulation, HLH, Inmunology, inmunodeficiencias, citotoxic T cells

Primary immunodeficiencies (PIDs) are a group of rare diseases that predisposes not only to infection but to autoimmunity, inflammation and cancer. A classical definition of PIDs applies for that condition that result from inborn-genetic errors and predisposes to multiple infectious diseases. However in the last 20 years, the clinical description of unnoticed phenotypes that predispose otherwise to healthy individuals to single infection has lead to a new definition of PIDs as a single-gene inborn errors of immunity that not necessarily predispose to multiple infections but a narrow spectrum of specific infections, limited to one microbial genus or even single species. Among these new group of Mendelian diseases, is that condition that predispose to fatal infections by Herpes virus such as Epstein-Barr Virus (EBV). There are two mayor phenotypes associated with fatal infections with EBV that are the result of single-gene errors. In the first group, the emergence of malignancy such as lymphoproliferative syndromes is the hallmark of the disease, whereas in the second group, the development of Hemophagocytic lymphohystiocytosis (HLH) is the manifestation distinctive. For XLP syndromes there are three major genes identified hitherto: SH2D1A, BIRC4 and ITK. HLH is a dysregulation of the immune response that results from an excessive activation of macrophages as consequence of a storm of pro-inflammatory cytokines. When a genetic cause underlies the development of HLH, this disease is also known as FHL (Familiar lymphohystiocytosis). Depending of the genetic cause, there are five forms of HLH. For FHL-1, there is no gene identified. FHL2 is due to mutations on PRF1, the gene that encodes for perforin. In FHL3 is the result of mutations in UNC13D the gene that encodes for Munc 13-4. FHL4 is the consequence of mutations in STX11, the gene responsible for Sintaxin 11. Mutations in STXBP2, the gene that encodes for Munc 18-2, are responsible for (FHL5). HLH is also associated to three syndromes that result in primary immunodeficiency. They include Griscelli Syndrome, Chediak Higashi Syndrome and Hermansky-Pudlak syndrome, which are due to mutations in

RAB27a, LYST, and AP3B1 respectively. All genes describe as responsible for familiar forms of HLH and syndromes associated encodes for proteins that are participate in vesicular traffic of cytotoxic granules of are components of cytotoxic machinery. In addition, patients with lymphoproliferative syndromes may also develop HLH. The molecular or genetic diagnosis of patients with HLH represents an important challenge for physicians because there is at least nine different genes that may explain the emergence of HLH. In order to favor the identification of mutations in genes that result in fatal infections to Herpes virus, we have develop a molecular algorithm that have been used in previous studies. The algorithm is based on functional assays that asses the degranulation response of cytotoxic cells and the expression of the listed proteins. An advantage of this algorithm is that all studies are based on the use of flow cytometry which allow to obtain diagnosis in less than 72 hours from the moment that sample is received. In addition, our objective is also to provide physicians a better mechanism to get a molecular diagnosis and to apply better therapies. To date, we have applied the algorithm in samples from 6 patients. The results suggest a possible FHL diagnosis for two patients. We found reduced cytotoxicity in two patients with likely Chediak-Higashi syndrome and a reduced cytotoxicity in a patient with likely Griselli syndrome. In one patient, a decreased perforin expression was also observed. Altogether, our results suggest that the implementation of this type of analyses represents a potential tool for a rapid differential diagnosis that could indicate the altered pathway and finally lead to the identification of mutations in specific genes.

CD43 processing in T lymphocytes

Angel Francisco Flores-Alcantar[1,2], Fernando Emmanuel Pedroza-Ibarra[1,2], Yvonne Rosenstein[1,2]

[1]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
[2]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
alcantar@ibt.unam.mx; fpedroza@ciencias.unam.mx; yvonne@ibt.unam.mx

Keywords: Sialomucins, T cells, TLR, Signal pathways, CD43

CD43 is a highly glycosylated protein localized on the membrane of lymphohematopoietic cells except for naive resting B cells and erythrocytes. The extracellular domain has a rigid rod-like shape that extends about 45nm from the cell surface. Similar to other mucins and sialomucins, the ectodomain of CD43 is bulky and negatively charged due to the abundant O-linked carbohydrate chains decorated with terminal sialic acids residues, possibly accounting for the anti-adhesive function of CD43. We have shown that in T lymphocytes, CD43 is a co-receptor molecule that signals independently of the TCR and CD28, a function that requires the highly conserved cytoplasmic tail of the molecule. Our group and others have shown that CD43-mediated signals alone or in combination with those of the TCR regulate signal pathways involved in cell functions such as proliferation, differentiation, migration, survival, adhesion...

Interestingly, CD43 has been reported to be down-regulated from the surface of human neutrophils after TNF-α treatment, through a proteolytic-dependent process. Also, in a colon tumor cell line, the intracytoplasmic tail of CD43 has been found to interact with beta-catenin and migrate to the nucleus, further confirming the processing of this molecule. In the present work, we evaluated whether the regulation of the expression level of CD43 also occurred in T lymphocytes in response to different stimuli. We will show data indicating that in human T lymphocytes stimulation through TLRs or TCR and CD28 ligation leads to a decrease in the expression level of CD43. The preliminary results suggest that the processing of this sialomucin may be dependent on the activity of metalloproteases and gamma-secretase. Altogether, our data indicate that in addition to participating in T cell activation, CD43 is processed in response to TCR and CD28- or TLR-mediated signals, underscoring novel signaling pathways through which CD43 regulates T_cell function.

Funded by PAPIIT/DGAPA, UNAM and CONACYT, MEXICO

Analysis of the putative interaction sites within the cytoplasmic tail of CD5 with RAS GAP, a negative regulator of TCR signaling during T cell development

Stephani Margarita Quiñones Lara[1], Carlos Mier Aguilar[2], Lorenza María Jaramillo[1], Adriana Rodriguez[1], Francisco Lozano[3], Gloria Soldevila[4], Nelly Stella Roa Molina[1]

[1]Centro de investigaciones Odontológicas, Pontificia Universidad Javeriana, Bogotá, Colombia
[2]Instituto Nacional de Enfermedades Respiratorias. Departamento de Investigación en Enfermedades Infecciosas (CIENI), Mexico, Mexico
[3]Departament de Cirurgia, Facultat de Medicina, Universitat Autònoma de Barcelona, Barcelona, Spain
[4]Departamento de Inmunología, Instituto de Investigaciones Biomédicas, UNAM, Ciudad de México, México, Mexico, Mexico
quinoness@javeriana.edu.co; cama.mier@gmail.com; lorenzaj@javeriana.edu.co; arodrig@javeriana.edu.co; flozano@clinic.ub.est; soldevi@unam.mx; nelly.roa@javeriana.edu.co

Keywords: Tyrosine, CD5, Signaling Pathways, Thymocyte selection, RasGAP

Background: T cell development occurs in the thymus as a result of the recognition of self-peptide/MHC complexes expressed on stromal cells by TCR expressed on developing thymocytes. TCR Signal strength play a role in positive versus negative selection, which finally results in the selection of a immune competent, but auto-tolerant T cell repertoire. We have previously demonstrated that CD5, a 67KDa surface receptor expressed on thymocytes, modulates is T cell selection, by down regulating TCR and as a results, rescuing from apoptosis those thymocytes expressing high affinity/aviditiy TCR and promoting their selection, which include natural T regs.

We have previously reported that the absence of the pseudo-ITIM (Y429-Y441)domain in CD5 leads to upregulation of the ubiquitin ligase c-Cbl. We proposed that the molecular mechanisms underlying CD5-mediated TCR down regulation may involve the association of negative regulators such as RasGAP, a Ras GTPase, that was shown to associate with phosphopeptides containing the tyrosine 429 (Y429) in pervanadate stimulated thymocytes, This opened the possibility that RasGAP might be recruited to CD5 through the pseudo ITIM motif (Y429-Y441) of CD5 during thymocyte development. However, to date there is no in vivo evidence demonstrating the association of RasGAP to these residues or to other CD5 potential phosphorylation sites such as Y463 in thymocytes.

Rationale

To understand the molecular mechanisms by which RASGAP modulates T cell development through CD5 has great biological relevance, as it has shown that acute lymphoblastic leukemia of T lymphocytes (T-ALL), can triggered by an increase in the activation of Ras, which may o not be related with association of RASGAP with CD5.

Objective: To investigate the recruitment of RasGAP to the CD5 cytoplasmic tail and identify the putative tyrosine residues involved in this association in murine thymocytes.

Methodology: Murine thymoma EL-4 cells were stably transfected with wild type (WT) human CD5 molecule or recombinant hCD5 containing point mutations from Y to A, in specific tyrosine residues: Y429 and triple mutant Y429/YY441/Y463. EL-4 stable clones were stimulated with biotinylated antibodies α-mCD3, α-mCD5 and α-hCD5, cross-linked with streptavidin or stimulated with pervanadate as a control. Cells were lysed with NP-40 and immunoprecipitated with anti-RasGAP and proteins were separated by electrophoresis in polyacrylamide gels (SDS-PAGE) and transferred to the PVDF membrane. Western blot analysis was performed with anti phosphotyrosine antibody (4G10) and polyclonal anti-human CD5 to analyze co-immunoprecipitated proteins.

Results: In EL4-WTCD5 EL4 cells anti-RasGAP was able to immunoprecipitate hCD5, both under basal conditions as well as after anti-CD3+ anti CD5 crosslinking or pervanadate treatment, indicating that RASGAP can be constitutively associated to the cytoplasmic tail of CD5. This association was not apparently enhanced under any stimulatory conditions. To identify the putative residues involved in this association, analysis of mutant clones was performed. Interestingly, the triple Y mutant, lost the ability co-immunoprecipitate CD5 with RasGAP, suggesting that Y residues are important for its recruitment under basal conditions. However, under stimulation conditions RasGAP wasn only to immunoprecipitate CD5 after CD3 plus CD5 co-crosslinking, but not after CD3 crosslinking alone, suggesting that under these conditions, RasGAP could be recruited to CD5 through other residues, such as serine or threonine residues.

On the other hand analysis of the Y429 mutant indicated that although this residue was hypothesized to be the putative binding residue for RasGAP, in the absence of this tyrosine, hCD5 was co-immunoprecipitated with RasGAP, both under basal conditions or after CD3 plus CD5 crosslinking. These data suggest that Y429 is not

required for the association of RasGAP to CD5, contrary to the reported biochemical data describing the binding of RasGAP to the phosphorylated pseudo ITIM domain. Finally in this mutant RasGAP co immunoprecipitated with a protein of approximately 80KDa, which may represent p85 subunit of PI3K which can be recruited to CD5 after Lck phosphorylation. As Y441 is not a target for Lck, this result suggests that as we previously proposed, that Y463 present in the carboxy-terminal region of CD5, may be responsible for the recruitment of PI3k to CD5.

In summary, we demonstrate RasGAP is constitutively recruited to CD5 through a non tyrosine residue. Therefore the putative binding domain of RasGAP to CD5 (Y429) is dispensable for its association with CD5, and suggests that the pseudo ITIM domain, previously demonstrated to downregulate TCR signaling may required the association with other potential negative regulators such as SHP-1, which could be recruited to Y429 upon tyrosine phosphorylation by Lck.

This work was supported by Academic Vicerrectry of Pontificia Universidad Javeriana.

New effective therapeutic approaches to control neuroinflammation improve several neuropathologies

Gabriela Meneses[1], José Alejandro Espinosa[1], Dunia Rassy[1], José Alfredo Esteban[1], Nicolás Pérez[1], Brandon Barcena[1], Guillermo Olvera[1], Esperanza García[2], Hugo Bessedovsky[3], Gladis Fragoso[1], Edda Sciutto[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Instituto Nacional de Neurología y Neurocirugía (INNN), Mexico City, Mexico
[3]Department of Experimental and Clinical Biopsychology, Philipps University of Marburg, Marburg, Germany
quilaz@yahoo.com; alexec2803@gmail.com; dnax100@gmail.com; ppfredd@gmail.com; nicolas2309@outlook.es; bmbc1520@gmail.com; feuer.olgr@comunidad.unam.mx; egarciainnn@gmail.com; besedovs@mailer.uni-marburg.de; gladisfragoso@hotmail.com; edda@uanm.mx

Keywords: Neuroinflammation, glial cells, neuropathologies, Intranasal glucocorticoids, Electric nerve stimulation

Abstract

Neuroinflammation is a complex phenomenon that could be triggered by a local or a systemic injury, or even by an exacerbated systemic inflammation. Acute neuroinflammation (NI) is a beneficial defensive response to harmful stimuli essential to maintain the homeostasis in the brain. In contrast, when sustained, NI can lead to pathological conditions.

NI is a common trait in many nervous system disorders that may promote their onset and progression. Its role in sepsis, Parkinson disease, stroke, and multiple s clerosis places NI as a new common therapeutic target for pharmacological interventions. Controlling subacute and chronic NI may help to restore CNS physiology and homeostasis.

Glucocorticoids (GC) are widely used for the control inflammation in chronic inflammatory diseases. However, their use is much more limit to control NI due to the high doses required to reach therapeutic central doses that also result in metabolic non desirable side effects.

The efficiency to control NI of two novel strategies: electric stimulation of the vagus nerve (ESVN) and intranasal administration of GC was confirmed in a murine model of septic shock induced by intraperitoneal administration of LPS.

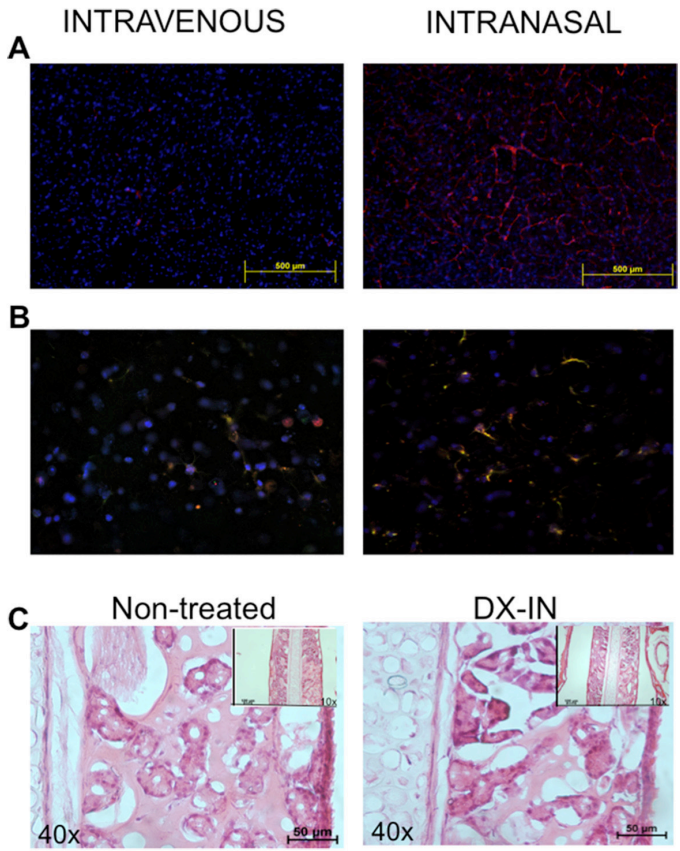


FIGURE 1: Glucocorticoids (GC) intranasally administered can directly reach the CNS via the axons of millions of olfactory nerve neurons that cross the upper posterior nasal mucosa. They can also cross the mucosa to reach axons of the trigeminal nerve, reaching the SNC parenchyma without crossing the Blood Brain Barrier (BBB). The higher detection of GC labeled with Alexafluor 594 (red) in the CNS cortex when intranasally is shown in Fig. 1A. GFAP labeled with Alexafluor 488 (green) was co-localized in astrocytes detected in yellow (Fig.1B). No histological damage was detected in the nasal epithelium of mice intranasally treated with GC (Fig.1 C).

Thereafter, the efficacy of ESVN to control NI and restore dopaminergic neurons in the substantia nigra pars compacta was experimentally demonstrated in experimental model of Parkinson induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Furthermore, intranasal of GC more effectively than the intravenous administration control the neurological symptoms, neural damage in the murine autoimmune encephalomyelitis elicited by the myelin oligodendrocyte glycoprotein MOG35-55 peptide, the most widely used model of multiple sclerosis. A single dose of GC intranasally administered in mice in which the middle cerebral artery was occluded for one hour significantly prevent mice mortality, morbidity, NI, neural damage and reduced the size of the ischemic lesions.

In respect to NI control, both treatments effectively modulate pro-inflammatory. ESVN reduced central levels of TNFα, IL-1β, and IL6 (measured by ELISA) and microglial activation (Iba-1+ and CD45+/CD11b+) (measured by IFC, WB and FC), in the cortex and hippocampus. Meanwhile, intranasal GC reduced central levels of IL6 (measured by ELISA), astrocytes activation (GFAP+) (measured by IFC) and the percentage of CD11b+/CD45+/Ly6G+ infiltrated neutrophils (measured by FC).

In summary, our results highlight the efficacy of ESVN and intranasal GC of reducing Neuroinflammation in several neuropathologies, restoring homeostasis more efficiently than current available treatments.

The antagonic effects of the tumor necrosis factor and the brain derived neurotrophic factor on cholinergic neuritogenesis and viability

Alejandro Ramirez, Leonor Pérez-Martínez, Gustavo Pedraza-Alva

Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
alex_ramirezolv@hotmail.com; leonor@ibt.unam.mx; gustavo@ibt.unam.mx

Keywords: Amyloid beta-Peptides, BDNF, Neuroinflammation, cholinergic, Cell viability, Neurite outgrowth, TNF-α, SN56 cell line

Alzheimer disease (AD) was first characterized in 1903 by Alois Alzheimer, where he described progressive memory loss and disorientation, later on, in post mortem brain slices the histopathological markers of the disease were identified, which we now know as the senile plaques with the β-amyloid peptides at its core, and the neurofibrillary tangles formed by the hyperphosphorylation of Tau protein (Hippius and Neundörfer 2003). In recent years it has been widely demonstrated that the accumulation of β-amyloid peptides can trigger an inflammatory process with neurodegenerative consequences in the brain, mostly affecting important regions that are essential for cognitive functions and memory like the hippocampus and the cortex.

Today it is well accepted that the production of inflammatory cytokines, initiated by the recognition of β-amyloid peptides by the microglia, play a key role in the development of the neuropathophysiology of AD. The plasmatic and cerebrospinal fluid levels of Tumor Necrosis Factor (TNF), and other inflammatory cytokines, are higher in mild cognitive impairment (MCI) and late onset AD patients, than the levels of healthy individuals (Chen et al. 2012)(Álvarez et al. 2007). Interestingly, the use of monoclonal antibodies directed against TNF in APP/PS1 AD transgenic mice models show a 40 to 60% reduction of senile plaques containing β-amyloid peptides and a reduction in Tau hyperphosphorylation, 3 to 14 days after intracerebroventricular (ICV) injection (Shi et al. 2011). Additionally, perispinal administration of etanercept, which blocks the binding of TNF to its receptor, improves the cognitive capacities of AD individuals (Tobinick and Gross 2008). Thus, suggesting that the loss of cognitive functions and neurodegeneration in AD is in part due to the constant presence of TNF.

Even though microglia are responsible for the release of proinflammatory cytokines that alter neuronal function, these cells also release a number of factors that promote neuronal survival. One of these factors found in areas of the brain with high synaptic plasticity is the Brain Derived Neurotrophic Factor (BDNF), which promotes neuron development, differentiation and plasticity (Leal et al. 2015). However, in post-mortem

studies performed in AD brains, BDNF immunoreactivity is decreased within neurons that have neurofibrillary tangles (Connor et al. 1997); furthermore, in AD brains there are increased levels of the truncated BDNF receptor TrkB (Connor et al. 1996), which lacks the phosphotyrosine domain. Thus, suggesting that in AD, inhibition of BDNF functions favors cognitive decline and memory loss. In accordance with this, in vitro and in vivo studies have shown that administration of exogenous BDNF can prevent neuronal cell death. In primary rat cortical cultures, BDNF protects against the toxic effects of βA42 (Arancibia et al. 2008). Furthermore, the administration of BDNF in AD animal models can prevent cell death and neuronal atrophy, thus preventing cognitive deterioration (Nagahara et al. 2009). Thus, BDNF has been proposed as treatment for diverse neurodegenerative processes.

Altogether, these experimental evidences suggest that in early stages of AD development, BDNF levels are enough to counteract the neurotoxic effects that inflammatory cytokines like TNF elicit, but as the disease progresses and more inflammatory cytokines are released in response to βA, they block the neuroprotective effects of BDNF, resulting in the loss of cognitive function and neurodegeneration. Thus, the balance between the signals generated by inflammatory cytokines that promote cell death and the neuroprotective signals generated by BDNF is lost in advanced stages of Alzheimer's disease. Therefore, we evaluated if the signals generated by TNF interfere with the signaling pathway of BDNF regulating neuronal survival and neurite outgrowth in cholinergic neurons.

Here we show that BDNF can promote SN56 cholinergic neurite outgrowth in a concentration and time dependent manner and that, as expected, TNF promotes a significant reduction in cell viability. When SN56 neurons were incubated with TNF in the presence of BDNF, the protective effects of BDNF were counteracted by the cytokine in a concentration dependent manner. Even in the presence of high concentrations of BDNF, TNF decreased cholinergic neurons viability.

In addition to affecting cell viability, TNF also prevented BDNF-induced neurite outgrowth. TNF treatment decreases both maximum neurite length and the number of neurites per cell compared with those from cells treated with BDNF alone.

Together, our results indicate that TNF blocks the BDNF neuroprotective and synaptogenic functions in cholinergic neurons. Whether TNF impairs BDNF functions through a direct negative effect on the mechanism controlling the neuronal survival and neurite growth or indirectly by blocking the TrkB signaling pathway in cholinergic neurons, is currently under investigation.

REFERENCES

Álvarez, Antón, Ramón Cacabelos, Carolina Sanpedro, Manuel García-Fantini, and Manuel Aleixandre. 2007. "Serum TNF-Alpha Levels Are Increased and Correlate Negatively with Free IGF-I in Alzheimer Disease." Neurobiology of Aging 28 (4):533–36. https://doi.org/10.1016/j.neurobiolaging.2006.02.012.

Arancibia, S., M. Silhol, F. Moulière, J. Meffre, I. Höllinger, T. Maurice, and L. Tapia-Arancibia. 2008. "Protective Effect of BDNF against Beta-Amyloid Induced Neurotoxicity in Vitro and in Vivo in Rats." Neurobiology of Disease 31 (3):316–26. https://doi.org/10.1016/j.nbd.2008.05.012.

Chen, Rui, You Yin, Zhongxin Zhao, L Huang, and Shuqi Huang. 2012. "Elevation of Serum TNF-α Levels in Mild and Moderate Alzheimer Patients with Daytime Sleepiness." Journal of 244 (1–2). Elsevier B.V.:97–102. https://doi.org/10.1016/j.jneuroim.2011.12.015.

Connor, B, D Young, P Lawlor, W Gai, H Waldvogel, R L Faull, and M Dragunow. 1996. "'Trk Receptor Alterations in Alzheimer's Disease." Brain Research. Molecular Brain Research 42 (1):1–17. https://doi.org/10.1016/S0169-328X(96)00040-X.

Connor, B, D Young, Q Yan, R L Faull, B Synek, and M Dragunow. 1997. "Brain-Derived Neurotrophic Factor Is Reduced in Alzheimer's Disease." Brain Research. Molecular Brain Research 49 (1–2):71–81. https://doi.org/10.1016/S0169-328X(97)00125-3.

Hippius, Hanns, and Gabriele Neundörfer. 2003. "The Discovery of Alzheimer's Disease." Dialogues in Clinical Neuroscience 5 (1):101–8. https://doi.org/10.1055/s-2008-1026558.

Leal, Graciano, Pedro M. Afonso, Ivan L. Salazar, and Carlos B. Duarte. 2015. "Regulation of Hippocampal Synaptic Plasticity by BDNF." Brain Research 1621. Elsevier:82–101. https://doi.org/10.1016/j.brainres.2014.10.019.

Nagahara, Alan H, David A Merrill, Giovanni Coppola, Shingo Tsukada, Brock E Schroeder, Gideon M Shaked, Ling Wang, et al. 2009. "Neuroprotective Effects of Brain-Derived Neurotrophic Factor in Rodent and Primate Models of Alzheimer's Disease." Nature Medicine 15 (3):331–37. https://doi.org/10.1038/nm.1912.

Shi, Jian-Quan, Wei Shen, Jun Chen, Bian-Rong Wang, Ling-Ling Zhong, Yin-Wei Zhu, Hai-Qing Zhu, Qiao-Quan Zhang, Ying-Dong Zhang, and Jun Xu. 2011. "Anti-TNF-α Reduces Amyloid Plaques and Tau Phosphorylation and Induces CD11c-Positive Dendritic-like Cell in the APP/PS1 Transgenic Mouse Brains." Brain Research 1368. Elsevier B.V.:239–47. https://doi.org/10.1016/j.brainres.2010.10.053.

Tobinick, Edward L, and Hyman Gross. 2008. "Rapid Improvement in Verbal Fluency and Aphasia Following Perispinal Etanercept in Alzheimer's Disease." BMC Neurology 8 (1):27. https://doi.org/10.1186/1471-2377-8-27.

Understanding the autophagy-inflammation correlation in the Alzheimer´s disease development

Martha Pedraza-Escalona[1], Lourdes Alvarez-Arellano[2], Tonali Blanco-Ayala[1], Nohemi Camacho-Concha[3], Javier Cortés-Mendoza[1], Leonor Pérez-Martínez[1], Gustavo Pedraza-Alva[1]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Hospital Infantil de México Federico Gómez, Mexico City, Mexico
[3]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
mapedmx@yahoo.com; lourdes.alvareza@gmail.com; tblanco@ibt.unam.mx; adriana.nozel@gmail.com; cjcm@ibt.unam.mx; leonor@ibt.unam.mx; gustavo@ibt.unam.mx

Keywords: Caspases, Inflammasomes, Inflammation, Alzheimer's disease, 5XFAD mice, autophagy dysfunction

Background: Autophagy is a cellular mechanism that, in physiological conditions, promotes proteolytic degradation of organelles and protein aggregates at the lysosome vesicles. This process allows the cellular recycling and cleaning. Autophagic alterations have been directly related to several neurodegenerative diseases. Alzheimer´s Disease (AD), the first cause of dementia around the world, has two hallmarks involved with the accumulation of protein aggregates: extracellular β-amyloid plaques and intracellular neurofibrillary tangles of the hyperphosphorylated tau protein. Interestingly, several reports have indicated a correlation between the autophagy and the inflammatory process elicited by invasive pathogens and metabolic diseases. The innate immune receptors can positive or negatively regulate autophagy depending of the cellular context (Lapaquette et al., 2015). In fact, the intracellular NOD like receptors (NRL receptors), which form the multiprotein oligomer called inflammasome, can negatively regulate the autophagy (Jounai et al., 2011). Nevertheless, François et al., (2014) reported that the inflammation could induce autophagy in an older transgenic mice model of the AD. Thus, the relationship between the inflammation process and autophagic flux on AD development remains largely unclear. Here we evaluated whether the inflammatory response induced by the β-amyloid plaques impairs memory via disrupting the autophagic flux in the 5XFAD mice model of the AD.

Methods: The 5XFAD (C57BL/6XSJL) mice were maintained as heterozygous by crossing with C57BL/6XSJL F1 mice. The C57BL/6XSJL were used as wild type. To obtain 5XFAD-caspase-1 knockout (KO) mice, the 5XFAD mice were crossed with caspase-1 KO (C57BL/6X129S), the 5XFAD/caspase-1 heterozygous were back-crossed with

caspase-1 KO mice, the caspase-1 KO littermates from these crosses were also used. At least five males/group from 12-, 20- and 32-week-old were used. Morris water maze test was used to determine cognitive defects. After perfusion, 30 micrometers-thick sagittal cryostat sections of the brains were used to determine the amyloid plaques quantification by Thioflavin S staining and the presence of active astrocytes (GFAP), neurons (NeuN) and a marker of autophagy (LC3) by immunofluorescence confocal microscopy. Additionally, the ultrastructural mitochondrial morphology was analyzed by electron microscopy. In the same way, tissue homogenates from prefrontal cortex and hippocampus were used to analyze the presence of amyloid deposition, LC3, p62, Ubq, p-AKT, AKT, p-AMPK, AMPK. Analysis of the expression of CD86 and TNF was determined by qPCR. Data are presented as mean ± SEM and were statistically analyzed by one-way ANOVA followed by Tukey's post hoc test or two-way repeated measures analysis of ANOVA followed by Newman-Keuls multiple comparison's post-hoc test or Sidak's multiple comparisons tests. $P < 0.05$ was considered statistically significant.

Results: The 5XFAD/caspase-1 deficient mice showed increased learning ability and memory compared with 5XFAD mice at 12- and 20-week-old. This correlated with the fact that 5XFAD mice lacking caspase-1 showed a decreased β-amyloid plaque number in the cortex and the hippocampus, as well as reduced oligomeric β-Amyloid peptide levels independently of the ages analyzed. Furthermore, the 5XFAD lacking caspase-1 showed attenuated astrogliosis and pro-inflammatory markers (CD86 and TNF) in the prefrontal cortex compared with those 5XFAD mice. Additionally, a reduced accumulation of mitochondria with aberrant morphology was observed in the cortex of the 5XFAD lacking caspase-1 mice in counterpart with 5XFAD transgenic mice. We found that caspase-1 deficiency attenuated the alteration in autophagosomes as well as the reduction in the number of autolysosomes observed in the cortex of the 5XFAD mice. This correlated with a decrease in the percentage of LC3 positive cortical neurons and decreased number of LC3 puncta per neuron in the 5XFAD caspase-1 deficient mice. At the molecular level, we found that the increased levels of LC3-II isoform, oligomerized p62, as well as ubiquitylated proteins, observed in the hippocampus of the 5XFAD mice from 20-week-old, were reduced to wild type levels in the 5XFAD-caspase-1 deficient mice. Moreover, the increased phosphorylayed AKT levels as well as the reduction of phosphorylated AMPK levels were restored in the hippocampus of the 5XFAD/caspase-1 deficient mice to levels observed in the hippocampus of wild type animals. Together, these results indicate that caspase-1-dependent inflammation resulting from β-amyloid peptide accumulation alters brain autophagy resulting in memory loss. In accordance with previous observations showing that the autophagy loss promotes neurodegeneration in vivo (Komatsu et al., 2006).

Conclusions: Our results indicate that inflammation triggered by inflammasome activation favors β-amyloid load and plaques formation, not only through diminishing microglia phagocytic activity (Koenigsknecht-Talboo J and Landreth, 2005), but also impairing autophagy flux in neurons resulting in memory loss.

REFERENCES

Francois, A., Rioux Bilan, A., Quellard, N., Fernandez, B., Janet, T., Chassaing, D., . . . Page, G. (2014). Longitudinal follow-up of autophagy and inflammation in brain of APPswePS1dE9 transgenic mice. J Neuroinflammation 11:139.

Jounai N., Kobiyama K., Shiina M., Ogata K., Ishii K. J., Takeshita F. (2011) NLRP4 negatively regulates autophagic processes through an association with Beclin1. J Immunology. 186:1646–1655.

Koenigsknecht-Talboo, J., Landreth, GE. (2005). Microglial phagocytosis induced by fibrillar beta-amyloid and IgGs are differentially regulated by proinflammatory cytokines. J Neurosci. 25:8240–8249.

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., ... Tanaka, K. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441(7095):880–884.

Lapaquette, P., Guzzo, J., Bretillon, L., & Bringer, M. A. (2015). Cellular and molecular connections between autophagy and inflammation. Mediators Inflamm 2015:398483.

An enriched environment restores glucose homeostasis, reduces inflammation and modifies microRNA levels in the hypothalamus of obese mice

Sol Díaz de León-Guerrero[1], Karla Meza-Soza[1], Verónica Jiménez-Jacinto[2], Leticia Vega-Alvarado[1], Jonathan Salazar-León[1], Martha Pedraza-Escalona[1], Sara García[3], Diana Aguilar León[1], Alejandro Sanchez-Flores[1], Gustavo Pedraza-Alva[1], Leonor Pérez-Martínez[1]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Centro de Ciencias Aplicadas y Desarrollo Tecnológico, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[3]Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[4]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
mdiazdel@ibt.unam.mx; karla.mezasosa@childrens.harvard.edu; vjimenez@ibt.unam.mx; vegal@ibt.unam.mx; salazarj@ibt.unam.mx; mapedmx@ibt.unam.mx; saragarcia@uaem.mx; aguilarleon@hotmail.com; alexsf@ibt.unam.mx; gustavo@ibt.unam.mx; leonor@ibt.unam.mx

Keywords: Hypothalamus, Inflammation, Obesity, microRNA, Signaling Pathways

Obesity has become and increasing worldwide health issue over the past few years, where in some countries it accounts for almost one third of the adult population. Recent studies have shown that the development of obesity is accompanied by a chronic, low grade inflammatory state in different tissues, including adipose tissue, pancreas, and even the hypothalamus (Hotamisligil, 2006). In the brain, the hypothalamus is the structure that regulates food intake and energy expenditure to keep energy balance (Morton et al., 2014). Obesity induces the activation of inflammatory pathways in the hypothalamus, such as the IKK/NK-κB and the JNK pathway, that lead to the inhibition of insulin and leptin signaling which exacerbates the development of obesity (De Souza et al., 2005; Zhang et al., 2008). Accordingly, the inhibition of JNK1 or IKKβ in hypothalamic neurons leads to a reduction in food intake and weight gain, and increases insulin sensitivity (Posey et al., 2009; Sabio et al., 2010). Seeking different therapies that could reduce inflammation and activate hypothalamic function, here we studied the effects of an enriched environment in obesity. An enriched environment has been highly studied in different models because it leads to the activation of different brain regions, increases neurotrophin levels and enhances learning and memory (Nithianantharajah and Hannan, 2006; Praag et al., 2000). Additionally, an enriched environment is also capable of regulating the immune system, where it has been shown to alter cytokine levels and modulate the activation of different immune cells (Marashi et al., 2003; Xiong et al., 2014). Previous studies have shown that mice housed in an enriched environment

are leaner than mice housed in standard or control conditions. Furthermore, it has also been shown that an enriched environment can prevent weight gain in mice fed with a high fat diet by a process of fat "browning"(Cao et al., 2011). Therefore, for this study we sought to determine if an enriched environment could restore glucose homeostasis in mice that already present metabolic alterations produced by the administration of a high fat diet. C57BL/6 mice were divided into standard housing conditions and fed with either a normal diet or a high fat diet (HFD), after 13 weeks we confirmed that the mice fed with a HFD presented significant weight gain as well as glucose intolerance and insulin resistance. We then divided the mice to be maintained in the control housing condition or to be switched to an enriched environment while maintaining the same diet. We found that an enriched environment decreased basal glucose levels and increased glucose tolerance and insulin sensitivity, although it did not alter weight gain. In the liver we found that the enriched environment increased insulin signaling and decreased liver steatosis. We also observed reduced cell infiltration to the adipose tissue, a marker of inflammation. Using high throughput sequencing we found that the administration of a HFD and the exposure to an EE altered microRNA expression in the hypothalamus. Bioinformatic analysis predicted different microRNA targets that could be regulated in the insulin signaling pathway. Finally, we observed that an enriched environment reduced the levels of key inflammatory signaling proteins such as IKKβ, p65 and JNK; while it increased AKT phosphorylation in the hypothalamus. Together our results show that an enriched environment reduces the activation of different inflammatory pathways as well as alters gene expression to improve glucose homeostasis and insulin resistance in a model of obesity.

REFERENCES

Cao, L., Choi, E.Y., Liu, X., Martin, A., Wang, C., Xu, X., and During, M.J. (2011). White to Brown Fat Phenotypic Switch Induced by Genetic and Environmental Activation of a Hypothalamic-Adipocyte Axis. Cell Metab. 14, 324–338.

Hotamisligil, G.S. (2006). Inflammation and metabolic disorders. Nature 444, 860–867.

Marashi, V., Barnekow, A., Ossendorf, E., and Sachser, N. (2003). Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. Horm. Behav. 43, 281–292.

Morton, G.J., Meek, T.H., and Schwartz, M.W. (2014). Neurobiology of food intake in health and disease. Nat. Rev. Neurosci. 15, 367–378.

Nithianantharajah, J., and Hannan, A.J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat. Rev. Neurosci. 7, 697–709.

Posey, K. a, Clegg, D.J., Printz, R.L., Byun, J., Morton, G.J., Vivekanandan-Giri, A., Pennathur, S., Baskin, D.G., Heinecke, J.W., Woods, S.C., et al. (2009). Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. Am. J. Physiol. Endocrinol. Metab. 296, E1003-12.

Praag, H. Van, Kempermann, G., and Gage, F.H. (2000). Neural consequences of environmental enrichment. Nat. Rev. Neurosci. 1, 191–198.

Sabio, G., Cavanagh-Kyros, J., Barrett, T., Jung, D.Y., Ko, H.J., Ong, H., Morel, C., Mora, A., Reilly, J., Kim, J.K., et al. (2010). Role of the hypothalamic-pituitary-thyroid axis in metabolic regulation by JNK1. Genes Dev. 24, 256–264.

De Souza, C.T., Araujo, E.P., Bordin, S., Ashimine, R., Zollner, R.L., Boschero, A.C., Saad, M.J. a, and Velloso, L. a (2005). Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146, 4192–4199.

Xiong, K., Fan, C., Zhang, M., Shang, L., Cynthia, N., Li, Z., Yang, Z., Chen, D., and Huang, J. (2014). Short-term environmental enrichment exposure induces proliferation and maturation of doublecortin-positive cells in the prefrontal cortex. Neural Regen. Res. 9, 318.

Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H., and Cai, D. (2008). Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. Cell 135, 61–73.

IFN-γ induces a tolerogenic phenotype on macrophages/microglia during the chronic phase of experimental autoimmune encephalomyelitis

Juan Enrique Tichauer, Eric Acuña, Rodrigo Naves

Universidad de Chile, Santiago, Chile
jtichauer@gmail.com; hanamvericta@gmail.com; rnavesp@yahoo.com

Keywords: Microglia, Multiple Sclerosis, EAE, IFN-gamma, Tolerance induction

Microglia, in concert with infiltrating blood macrophages (dendritic cells) play a major role in the progression of Multiple Sclerosis. Their involvement in this chronic (relapsing) inflammatory disorder has been studied in the well-established murine model of experimental autoimmune encephalomyelitis (EAE). The inflammatory environment of EAE induces a microglial M1 phenotype characterized by the increase of MHC-II molecules and co-stimulatory molecules that locally induce the reactivation of effector-assisting T lymphocytes. In this study, we evaluated the effect of IFN-γ on the activation of macrophages/microglia during the chronic phase of EAE. Flow cytometry analysis of CNS infiltrating cells revealed that IFN-γ-treated EAE mice had a significantly lower frequency and absolute cell number of CD11b+ cells than PBS-treated control mice. Remarkably, IFN-γ-treated EAE mice had a significantly lower frequency of CD11b+CD45hi (activated) cells and a significantly higher frequency of CD11b+CD45low (resting) cells associated with higher expression of IL-10, compared with controls. Further analysis performed on the CD45hi subpopulation revealed that IFN-γ induced a decrease in macrophages (CD11b+CX3CR1midPD-L1hiCD45hi) expressing high levels of MHC-II molecules and co-stimulatory molecules without affecting activated microglia (CD11b+CX3CR1hiPD-L1loCD45hi). In addition, CD11b cells obtained from IFN-γ-treated EAE mice and pre-treated ex vivo with low doses IFN-γ/myelin peptide induced an increase on lymphocytes Tregs cells (Foxp3+/CD25hi) frequency. Overall, our results indicate that IFN-γ treatment induces a tolerogenic phenotype in macrophages/microglia during EAE effector phase.

Participation of the hypothalamic TRPV1 receptor in the reproductive axis

Pablo Nicolas Surkin[1,2], Ana María Genaro[3,4], Juan Carlos Elverdin[2], Andrea De Laurentiis[2,5], Javier Fernandez-Solari[1,2]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]Facultad de Odontología, Universidad de Buenos Aires, Buenos Aires, Argentina
[3]Instituto de Investigaciones Biomédicas (BIOMED UCA-CONICET), Buenos Aires, Argentina
[4]Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina
[5]CONICET Centro de Estudios Farmacológicos y Botánicos (CEFYBO), Buenos Aires, Argentina
nicolassurkin@hotmail.com; amgenaro@yahoo.com.ar; jelverdin@hotmail.com; andredelaurentiis@gmail.com; javierfsolari@yahoo.com.ar

Keywords: TRPV Cation Channels, GnRH, Kisspeptin, LPS, Reproductive axis, GnIH/RFRP3

It is known that immune challenge inhibits the hypothalamic pituitary gonadal (HPG) axis activity, and we have recently reported that the hypothalamic cannabinoid receptor type 1 (CB1) partially mediates this effect in male rats, favoring the control of inflammatory response in detriment of sexual hormone release. However, no studies evaluating the participation of TRPV1 vanilloid receptor, which also acts as a nonspecific receptor for the endocannabinoid anandamide, were reported. Therefore, we assessed the participation of the hypothalamic TRPV1 on the HPG axis activity in rats submitted or not to immune challenge induced by LPS. Male adult rats (n=6/group) were treated via intracerebroventricular (icv) with the TRPV1 antagonist Capsazepine (500ng/5ul), followed by an intraperitoneal (ip) administration of lipopolysaccharide (LPS, 5mg/kg) or saline 15 min later. 180 min post ip injections, hypothalamic pro-inflammatory cytokines and neuropeptides involved in reproductive function were assessed by qPCR. Hypothalamic Tnfα and Il1β mRNA expression was increased with both TRPV1 blockade and by LPS administration, separately ($p < 0.05$). Both experimental conditions also seem to inhibit the HPG axis by decreasing gonadotropin-releasing hormone (Gnrh) gene expression ($p < 0.05$). This disruptor effect was accompanied by decreased hypothalamic Kiss1 mRNA expression as well as by increased gonadotropin-inhibitory hormone (Rfrp3) mRNA ($p < 0.05$), neuropeptides regulating upstream GnRH synthesis and release, positively and negatively, respectively. Although hypothalamic TRPV1 blockade prevented the Kiss1 mRNA decrease induced by LPS, it did not show a significant effect on the expression of Rfrp3 or on that of Gnrh under the immune

challenge. In summary, our results suggest that unlike hypothalamic CB1 receptor, which mediates reproductive axis blockade during the immune challenge to prioritize homeostasis maintenance, TRPV1 receptors could act in physiological conditions, allowing the physiological response of the reproductive axis.

Effect of 17β-estradiol on oxidative stress and antioxidant activity in female mice infected with P. berghei ANKA

Jesús Aguilar Castro, Martha Legorreta-Herrera

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
jesus_aguilar_castro@yahoo.com.mx; marthal@unam.mx

Keywords: Malaria, Oxidative Stress, Antioxidant enzymes activities, 17B-estradiol, Superoxide Dismutase and catalase

Malaria is an infectious disease caused by parasites of the genus Plasmodium sp. On 2017, WHO reported 216 million of new cases and 445 thousand deaths around the world. Malaria exhibits sexual dimorphism, male show more severe pathology that females. Due to sexual hormones are responsible of the main differences among sexes it is likely sexual hormones are also involved in malarial sexual dimorphism. During malaria infections, the immune system control and eliminate Plasmodium via generation of oxidative stress. It is produced by cells such as macrophages and neutrophils whose function is to oxidize the main biomolecules that constitute the parasite. No specificity is the main disadvantage of oxidative stress, it doesn´t recognize between molecules from the parasite and from other cells, thus it must be closely regulated. Antioxidant enzymes are the most efficient via to regulate oxidative stress. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase are the main antioxidant enzymes and their determination is an indirect way to know the oxidant environment, in response to Plasmodium infection. Our group demonstrated that sexual hormones modify the immune system on malaria infections and that gonadectomy increases parasitemia, reduces the activity of antioxidant enzymes and enhances oxidative stress on CBA/Ca female mice. In other words, female hormones are related to Plasmodium elimination. Therefore, in this work we focus on the effect of 17β-estradiol, the main female hormone, on the oxidative stress and antioxidant activity in CBA/Ca female mice infected with P. berghei ANKA.

CBA/Ca female mice were separated in 4 groups of five mice: intacts (I), gonadectomized (Gx), treated with 17β-estradiol (E) or reconstituted with 17b-estradiol (GxE). Mice were gonadectomised, after a month of birth, when they reach the sexual maturity. One month later mice were treated with 12 μg de estradiol on 50 μL of sweet almond oil, used as vehicle. One day after last hormone administration,all mice were injected with 1000 erythrocytes infected with Plasmodium berghei ANKA. Parasitaemia was evaluated daily using blood smears Giemsa stained. On day eight post-infection mice

were sacrificed and blood, spleen, liver and brain tissues were collected to evaluate SOD, GPx and catalase activities as well as malondialdehyde levels (MDA) as an oxidative stress measure.

Gonadectomy significantly reduced the activity of SOD and GPx and increased slightly the MDA concentration on blood, meanwhile it reduced the activity of GPx and increased the MDA concentration on brain, compared to the intact group. The treatment with 17β-estradiol increased both the activity of catalase on blood and the activity of SOD on brain compared to the intact group. In addition, 17β-estradiol slightly reduced MDA concentration on liver. Reconstitution of gonadectomised female with 17β-oestradiol increased GPx activity and MDA concentration on blood and reduced the MDA concentration on liver compared to the Gx group.

We conclude that 17β-estradiol increases antioxidant activity of both catalase and SOD which reduces oxidative stress in brain. The 17β-estradiol main effects depend of both presence of the gonads and the particular tissue analysed.

Acetone fraction from the edible roots of Sechium edule (Jacq.) S.w. possess anti endothelial dysfunction activity

Celeste Trejo[1], Gabriela Castro[1], Marisol Mendez[1], Enrique Jimenez[2], José Pedraza[3], Omar Medina[3], Gerardo Arrellín[4], Alejandro Zamilpa[2], Joel Barrita[1], Beatriz Hernández[5], Christian Ramirez[6], Angelica Santana[7], Galia Lombardo[2], Gladis Fragoso[8], Gabriela Rosas[1]

[1]Facultad de Medicina, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[2]Centro de Investigaciones Biomédicas del Sur, Instituto Mexicano del Seguro Social, Xochitepec, Mexico
[3]Faculty of Chemistry, National Autonomous University of Mexico, Mexico City, Mexico
[4]Facultad de Ciencias de la Salud, Universidad Panamericana, Mexico City, Mexico
[5]Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[6]Facultad de Medicina, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[7]Centro de Investigación en Dinámica Celular, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[8]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
trejomc@hotmail.com; gcm_19@hotmail.com; mm.mary87@gmail.com; enriqueferrer_mx@yahoo.com; pedrazachaverri@gmail.com; omarnoelmedina@gmail.com; garrellin@up.edu.mx; azamilpa_2000@yahoo.com.mx; barritaj@hotmail.com; bhernandezt@hotmail.com; chari_ouka@hotmail.com; santana@uaem.mx; galia100000@yahoo.com.mx; gladis@unam.mx; gabyrosas62@hotmail.com

Keywords: Angiotensin II, Hypertension, Inflammation, Oxidative Stress, endothelial dysfunction, Sechium edule, vascular tissue remodeling

Introduction: Endothelial dysfunction (ED) is misbalance between the synthesis, release and/or effect of different factors of endothelial cells. It is indicative of vascular damage and plays a critical role in the pathogenesis of cardiovascular disorders like hypertension, renal dysfunction, and cerebrovascular diseases, which are leading causes of death worldwide (Schmitt and Dirsch, 2009). ED has a multi-factorial origin that may be associated with alterations in the concentration of glucose, triglycerides, LDL, VLDL, leptin, ROS, endothelin-1, Vascular Endothelial Growth Factor, C reactive protein, IL-6 and relevantly by Angiotensin II (AGII) (Adya et al., 2015; Husain et al., 2015; Jaimes et al., 2010; Liu et al., 2015) being liver and kidney the most affected organs (Bosch et al., 2010; Henke et al., 2007). It is characterized by a vasoconstrictor, pro-oxidative, pro-inflammatory, pro-thrombotic and proliferative status, along with vascular remodeling (Endemann and Schiffrin, 2004). A recent ethno medical survey of medicinal plants grown in Mexico revealed that Sechium edule (Jacq.) Sw. (Cucurbitaceae) is one of the most valued plants used for treating cardiovascular diseases, including hypertension (Argueta et al., 1994; Ibarra-Alvarado et al., 2010). The Fruits, young leaves, shoots,

stems and tuberous roots of the plant are edible. Because it was previously reported that it has antioxidant, anti-inflammatory and antihypertensive activities (Lombardo et al., 2014; Lozoya 1980), we wondered if it could control endothelial dysfunction.

Aim of the Study: To investigate the anti-endothelial dysfunction activity of the acetone fraction (rSe-ACE) obtained from the hydroalcoholic extract of the root in vivo.

Materials and Methods: The endothelial dysfunction was induced in female C57BL/6J mice with a daily intra-peritoneal injection of angiotensin II for 10 weeks. The rSe-ACE organic fraction or losartan as control, were coadministrated with the angiotensin for the same period. Arterial pressure was measured at 0, 5, and 10 experimental weeks. Kidney extracts were prepared to evaluate IL1β, IL4, IL6, IL10, IL17, IFNγ, TNFα, and TGFβ cytokines by ELISA and the pro-oxidative status by the activity of antioxidant enzymes. The expression of ICAM-1 was evaluated by immunohistochemistry in histological sections of kidneys. Kidney and hepatic damage, and vascular tissue remodeling were studied.

Results: The rSe-ACE fraction (10 mg/kg) was capable of controlling hypertension, as well as the pro-oxidative and pro-inflammatory status in kidney as efficiently as Losartan, bringing mice to normotensive levels. Additionally, it was more efficient than Losartan in avoiding liver and kidney damage. The phytochemical analysis characterization identified the cinnamic acid as major compound and linoleic, palmitic and myristic acids as the more abundant non-polar components in this mixture that has been reported to aid in control of hypertension, inflammation and oxidative stress, three important components of the endothelial dysfunction.

Conclusion: this study demonstrated, that rSe-ACE has anti-endothelial dysfunction activity in an experimental model and highlights the role of cinnamic acid and Fatty acids in the observed response.

REFERENCES

1. Adya, R., Tan, B.K., Randeva, H.S., 2015. Differential effects of leptin and adiponectin in endothelial angiogenesis. J. Diabetes Res. 2015:648239.
2. Argueta, V.A., Cano, A.L., Rodarte, M.E., 1994. Atlas de las Plantas de la Medicina Tradicional Mexicana. Instituto Nacional Indigenista; México. 1994 I:393-394

3. Bosch, J., Abraldes, J.G., Fernández, M., García-Pagán, J.C., 2010. Hepatic endothelial dysfunction and abnormal angiogenesis: new targets in the treatment of portal hypertension. J. Hepatol. 53(3):558-67.
4. Endemann, D.H., Schiffrin, E.L., 2004. Endothelial dysfunction. J. Am. Soc. Nephrol. 15(8):1983-92.
5. Henke, N., Schmidt-Ullrich, R., Dechend, R., Park, J.K., Qadri, F., Wellner, M., Obst M., Gross, V., Dietz, R., Luft, F.C., Scheidereit, C., Muller, D.N., 2007. Vascular endothelial cell-specific NF-kappaB suppression attenuates hypertension-induced renal damage. Circ. Res. 3;101(3):268-76.
6. Husain, K., Hernandez, W., Ansari, R.A., Ferder, L., 2015. Inflammation, oxidative stress and renin angiotensin system in atherosclerosis. World J. Biol. Chem. 26;6:209-17.
7. Ibarra-Alvarado, C., Rojas, A., Mendoza, S., Bah, M., Gutiérrez, D.M., Hernández-Sandoval, L., Martínez, M., 2010. Vasoactive and antioxidant activities of plants used in Mexican traditional medicine for the treatment of cardiovascular diseases. Pharm. Biol. 48(7):732–739. doi: 10.3109/13880200903271280.
8. Jaimes, E.A., Hua, P., Tian, R.X., Raij, L., 2010. Human glomerular endothelium: interplay among glucose, free fatty acids, angiotensin II, and oxidative stress. Am. J. Physiol. Renal Physiol. 298:F125-32. doi: 10.1152/ajprenal.00248.2009.
9. Liu, H., Chen, T., Li, N., Wang, S., Bu, P., 2015. Role of SIRT3 in Angiotensin II-induced human umbilical vein endothelial cells dysfunction. BMC Cardiovasc. Disord. 30:15-81.
10. Lombardo-Earl, G., Roman-Ramos, R., Zamilpa, A., Herrera-Ruiz, M., Rosas-Salgado, G., Tortoriello, J., Jiménez-Ferrer, E., 2014. Extracts and Fractions from Edible Roots of Sechium edule (Jacq.) Sw. with Antihypertensive Activity. Evid. Based Complement Alternat. Med. 2014:594326. doi: 10.1155/2014/594326.
11. Lozoya, X., 1980. Mexican Medicinal Plants Used for Treatment of Cardiovascular Diseases. Am. J. Chin. Med. 8(1-2):86-95.
12. Schmitt, C.A., Dirsch, V.M., 2009. Modulation of endothelial nitric oxide by plant-derived products. Nitric Oxide. 2:77-91.

Mycobacterium tuberculosis induces neuroinflammation and behavioral abnormalities during progressive pulmonary tuberculosis

Jacqueline Viridiana Lara Espinosa[1], Brenda Nohemi Marquina-Castillo[1], Luis Enrique Becerril Villanueva[2], Gilberto Pérez Sánchez[2], Mario Alberto Zetter[1], Ricardo Alberto Santana Martínez[3], Dulce Mata Espinoza[1], Jorge Alberto Barrios-Payan[1], Lenin Pavón Romero[2], Rogelio Hernandez Pando[1]

[1]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[2]Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz (INPRFM), Mexico City, Mexico
[3]Instituto Nacional de Neurología y Neurocirugía (INNN), Mexico City, Mexico
jvle_29@hotmail.com; brenda.marquina@gmail.com; lusenbeve@yahoo.com; gipersanchez@hotmail.com; zetter.salmon@gmail.com; santana_ricardo03@hotmail.com; dulmat@yahoo.com; qcjbp77@yahoo.com.mx; lkuriaki@gmail.com; rhdezpando@hotmail.com

Keywords: Behavior, Cell Death, Mycobacterium tuberculosis, neurotransmitter, Neuroinflammation; Cytokines

During the development of chronic inflammatory processes there is a complex activity of specific brain areas, producing neuronal activation and changes in the synthesis, production and activation of neurotransmitters and cytokines that in addition to contributing to efferent responses that regulate inflammatory and immune responses, may also affect neuropsicoaffective functions such as learning ability, memory and mood. Pulmonary TB is an infectious disease that brings about chronic inflammation and it may be conceivable that these neuroimmunological changes also may occur in the absence of brain infection. The aim of this study was to determine the inflammatory and local immune response as well as morphological, biochemical and behavioral abnormalities in the CNS in a mouse model of progressive pulmonary TB. The animals were intratracheally infected with 250,000 UFC of the strain H37RV and a control group received a saline solution. Cytokines were measured by RT-PCR in the cerebellum, hippocampus, and hypothalamus of each animal; neurotransmitters were quantified by HPLC and the effect of infection on the behavior was established by different behavioral tests. Finally, the histological damage was determined by light and electron microscopy. Our results show that infected animals display an increase in the hypothalamus, hippocampus and cerebellum of proinflammatory cytokines TNFa, IFNg and IL-12, IDO and iNOS enzymes and IL10, IL4 and TGFb. Depressive-like behavior, sickness behavior, anxiety-like behavior, neurological damage and an impairment not only in the short term memory but also in long-term memory were presented, which coincides

with changes in the neurotransmitters epinephrine, norepinephrine, dopamine and serotonin in the hypothalamus, hippocampus and cerebellum of infected animals. Neuronal death characterized by pyknotic nuclei and acidophilic cytoplasm in the hippocampus and frontal cortex was observed, as well as in epithelial cells of the choroid plexus. These results indicate that the extensive lung inflammation and cytokine production is associated with the presence in the CNS of cytokines, neuroinflammatory changes and production of neurotransmitters that could be the cause of neuronal death and behavioral abnormalities present in animals with pulmonary TB in the absence of culturable bacteria in the brain.

Contribution of vasopressin to pulmonary tuberculosis immunopathology

Mario Zetter-Salmón[1], Brenda Brenda Marquina[1], Dulce Adriana Mata[1], Jorge Alberto Barrios-Payan[1], Andres Quintanar-Stephano[2], Rogelio Hernandez Pando[1]

[1]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[2]Autonomous University of Aguascalientes, Aguascalientes, Mexico
zetter.salmon@gmail.com; brenda.marquina@gmail.com; dulmat@yahoo.com.mx; qcjbp77@yahoo.com.mx; aquinta@correo.uaa.mx; rhdezpando@hotmail.com

Keywords: Neuroimmunomodulation, Tuberculosis, Pulmonary, vasopressin, Pathogenesis, Lung immune responses

Tuberculosis (TB), the highly prevalent human disease caused by the intracellular pathogen Mycobacterium tuberculosis (Mtb) threats homeostasis at different levels, and besides its immunopathogenic hallmark, alterations in several hormones accompany and alters the course of infection. Thus, these endocrine changes have also an effect over innate and adaptive immune responses and, consequently, with the progression and fate of the disease. In this way, vasopressin (Avp) or antidiuretic hormone, an evolutionary well-conserved and stress-related nonapeptide produced mainly by hypothalamic neurons, is responsible of supporting some physiological phenomena related to inflammatory processes, like early changes in vascular permeability, macrophage activation and lymphocyte function, has also a role in tissue healing and has been related with inflammatory and autoimmune pathogenic processes. Moreover, vasopressinergic activity appears to be dyfunctional during human pulmonary TB.

The objective of this investigation is to determine whether Avp has a role in the pathogenesis of tuberculosis, using an experimental model of progressive pulmonary TB. Our results shown positive immunostaining to Avp in lung, particularly in macrophages with foamy morphology during the late phase of disease. Apparently these pulmonary macrophages are a source of the hormone. To study the possible contribution of this findings in pathogenesis, vasopressinergic activity was pharmacologically blocked during late stage of mice infection which resulted in less bacillary loads in lung of infected mice. Conversely, when a synthetic agonist was administered, more lung bacilli and fibrosis were noted.

Together, these results suggest that Avp could have an immunosuppressive/anti-inflammatory and profibrotic effect during late active TB. The specific effect of how Avp

interferes with immune mechanisms, such as antigenic presentation and phagocytic capacity of macrophages as well as in adaptative immune function is currently under research. Additionally, the pharmacological manipulation of vasopressin-receptor system could represent a new endocrine-immunotherapeutic strategy in infectious and inflammatory chronic diseases, particularly in TB, and blocking Avp could contribute to shorten the long antibiotic treatment. This interesting issue is also currently under research.

Vitamin D, Melatonin, Psychoneuro-endocrine-immunology and aging in Curitiba, Brazil

Dainery A Pérez Lastre[1], Miriam SJB Lastre González[2], Eduardo Santos Lima[3], Oliver Pérez[2,4]

[1]Hospital Vitória, Unidade Avançada São José Dos Pinhais, Rua Sete de Setembro, 290-Centro PR, Curitiba, Párana, Brazil
[2]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[3]Instituto de Saude Dr Santos Lima, Curitiba, Brazil
[4]Escuela Latinoamericana de Medicina, La Habana, Cuba
dperezlastre@gmail.com; oliver.perez@infomed.sld.cu; eduardosl@onda.com.br; oliverperez@giron.sld.cu

Keywords: Melatonin, Vitamin D, Skim elder, Psychoneuro-endocrine-immune, Eldeyer

Introduction: Skim aging is a natural process of a sequential phenomenon that occurs in the tissue and the organism as a whole during life which begins around 20 years and it is accelerated during menopause and androspause [1, 2]. On one hand, between 2000 to 2050 the Word Heath Organization (WHO) predicts that elder with more than 60 years will increases from 11 (~605 millions) to 21,5% (~2000 millions) and those with more than 80 years will be double with around 400 million3. Brazil is the 56/96 countries with a 12% and in 2025 will has the six population of elder of the world4 occupying the same position in Latin America5. Elderly has cause by endogen and exogenous factors. On the other hand, more than a half of world population has Vitamin D deficiency which is relevant in Curitiba associated with few sun shine present, because the main mechanism of production of Vitamin D is the exposition to sol6,7. On the other hand, Melatonin is the physiologic reparatory hormone. It is produce by the pineal gland during sleeping and extrapineally by different cells during the day. Its production is severely affected in adult live and particularly in elders. The Vitamin D and Melatonin interact with the psychoneuro-endocrine-immune suprasystem which controls the interactions between systems.

Aims: To look for the possible repercussion of Vitamin D and Melatonin over the skim aging at Curitibenses and to look for the mechanisms of Vitamin D and Melatonin with the psychoneuro-endocrine-immune suprasystem.

Results: There were found several papers about skim aging in the world and Brazil and Vitamin D; but lees of Melatonin and minor related with psychoneuro-endocrine-immune

suprasystem (Fig. 1, 2 and 3). The ageing occurs by intrinsic (thymus involution; replication senescence; chronic stimulation of the immune system, and hormonal changes) and extrinsic (ultraviolet radiation, tobacco and pollution) factors8,9,10. There are two types of Vitamin D, the colecalciferol (Vitamin D3) and the ergocalciferol (Vitamin D2) produce by animals and vegetal (mainly fungi and yeasts), respectively. Both are converted to calcitrol (1,25-dihidroxivitamin D), the active molecule, namely Vitamin D11. This is essential for multiples functions and the activation of more than 2000 genes functioning as a hormone through a specific receptor present in all body cells12,13,14. The main Vitamin D function is to maintain the homeostasis of phosphorus and calcium that guaranty the metabolism, the neurotransmission, and bon mineralization (15,16). The 80–80% of human Vitamin D come from the dermal synthesis induced by ultraviolet irradiation and the rest come from the diet17,18. The deficiency de Vitamin D is frequently. There is estimated that around one billion of person in the world has low level of this hormon19. The main symptoms and signs of the Vitamin

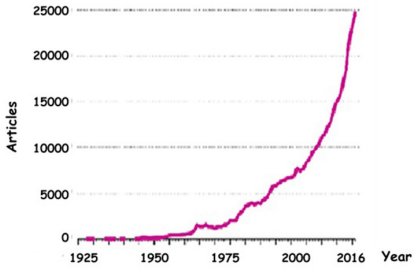


FIGURE 1: Number of articles of elder published in PubMed. Consulted 31.01.18

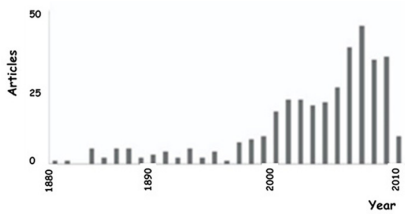


FIGURE 2: Number of articles of elder in Brazil published in LIACS. Consulted 31.01.18

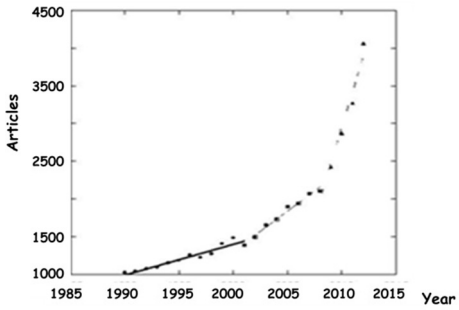


FIGURE 3: Number of articles of Vitamin D in Pubmed. Consulted 31.01.18

D deficit are: increase of diseases and infections, fatigue, bon pain, depression, healing problems, weight hair lost, and muscular pain20,21. The Melatonin (N-acetil-5-metoxi-tryptamina) is frequently present in nature since unicellular microorganism to human. It is produce in low quantity (serum <1 nM) by the pineal grand to go to systemic circulation and cerebrum where it is a chronobiology marker (maintain the biologic rhythm) and in higher quantity (tissue, 1–10 mM) extrapineally by several tissues and organs (retina, gastrointestinal tract, skin, bonne marrow, and lymphocytes) without circadian cycle. The latter is involved in mitochondrial homeostasis with antioxidants and anti-inflammatory activities. Both productions are decreasing with age22,23,24,25. Both Vitamin D and Melatonin are two supplementary hormones with diurnal and nocturnal cycles circadian, respectively. They are synthetized by the organism and acquired by alimentation or medication. There are four hormones of felicity: dopamine (main motivational regulator involved in obtain our goals); serotonin (promotion of pleasure sensation and wellbeing and its absence might cause depression); endorphin (pain and stress attenuation, liberated during physic exercise, it is consider as an natural analgesic and help to reduce stress and anxiety), and oxytocin (promote the confidence sensation, it is produce during childbirth, breastfeeding, and orgasm)26. The tryptophan and serotonin are precursors of Melatonin and in this way the felicity is related with serotonin presence. In addition, Vitamin D has also influence over the stress/depression process which is their main but not unique connection with the suprasystem. The low levels of Vitamin D are associated with a tendency to suffer irritability, depression, and rapid changes of humor. This may explain the relation Melatonin-Depression (Fig. 4). The stress is considering the siècle XXI disease8. The WHO estimates more than 300 million of persons living with depression, with an increase of more than 18% between 2005 e 201527. In 2017 near to 50 million in America will live with depression, near of 17% more than in 2005. The Brazilian and Curitibanenses are not escaping to this epidemic. In addition, the ACTH (adrenocorticotrophic hormone) is an important hormone for the secretion of adrenals corticosteroids (cortisol) which is involved in induction of immunosuppression during chronic stress8. Both Vitamin D and Melatonin act over: skim (production of Vitamin D and protecting the damage cause by UV light); bonne (paratiroides glands to regulate calcium production and small intestine for the calcium absorption); genes (stress oxidative and expression of mitochondrial proteins); nueroprotection (both); serotonin (production and action over receptor); immune system (both), respectively.

Conclusions: The Curitubenses received insufficient sol and the elders sleep less than others ages, by which their Vitamin D and Melatonin would be reduces and require diurnal and nocturnal supplementation or of their precursors, respectively. This is more hurry during the stress/depression process by which they need to be explored and act

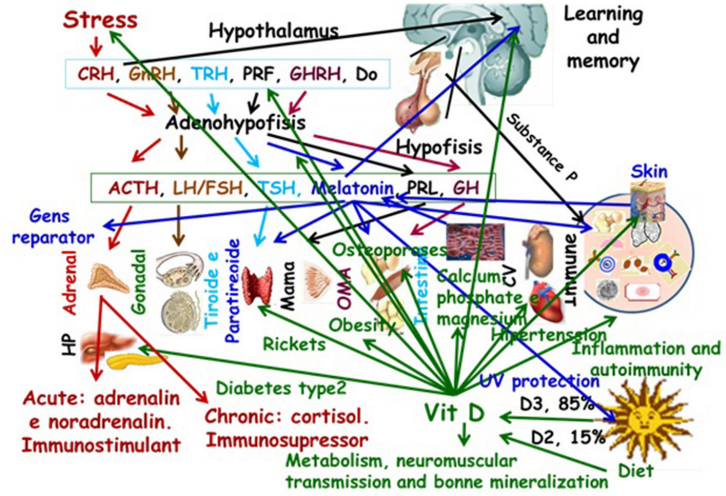


FIGURE 4: Vitamin D, Melatonin and Suprasystem interactions. Adapted of O Pérez e I Vega . Ecimed 2017

in concordance. We recommended practicing healthy habit of live, particularly making exercise, increment the diary exposition to sol and having good sleeping.

REFERENCES

1. Garcez CE, Arena-de-Souza R. Temas de Medicina Estetica, 3ª edição. 2011
2. https://www.linguee.pt/espanhol-portugues/traducao/envejecimiento+de+la+piel.html. Consulted 227.12.17
3. Índice Global de Envejecimiento, Age Watch 2015. Resumen ejecutivo. http://www.helpage.org/silo/files/gawi-2015-resumen-ejecutivo-.pdf. Consulted 28.12.17.
4. http://agencia.fapesp.br/brasil_tendra_la_sexta_poblacion_de_ancianos_del_mundo_en_2025/23653/. Consultado 26.12.17
5. Envejecimiento de la población: ¿está preparada América Latina? Editor. Daniel Cotlear, Primera edición en castellano: julio de 2009.
6. http://www.gazetadopovo.com.br/curitiba/curitiba-tem-menos-dias-de-sol-que-ny-londres-berlim-e-praga-97. Consulted 29.12.17.
7. http://www.bbc.com/portuguese/reporterbbc/story/2007/11/071108_vitaminaenvelhecimento.html. Consulted 29.12.17.
8. Pérez O e Veja I. Inmunología en el humano sano. Editora Ecimed 2017.
9. Alves R, Castro Esteves T y Trelles MA. Factores intrínsecos y extrínsecos implicados en el envejecimiento cutáneo. Cirugía Plástica Ibero-Latinoamericana. 2013; 39(1) Doi.org/10.4321/S0376-78922013000100013.

10. Flor J, Davalos M, Correa MA. Protetores solares. Química nova. 2007; 30(1), São Paulo.
11. http://www.misistemainmune.es/vitamina-d-y-el-sistema-inmune/. Consulted 5.1.18.
12. https://flaviopassos.com/2016/09/11/8-sinais-e-sintomas-de-deficiencia-de-vitamina-d-que-podem-estar-custando-caro-para-sua-saude/. Consulted 5.1.18.
13. S. Marín, L. Suárez, R. Díaz. Bases fisiológicas y estudio del metabolismo fosfocálcico. Revista de hormonas y factores de crecimiento, XIV (2011), pp. 3-9
14. H. Plotkin, F. Lifshitz. Rickets and osteoporosis. In: Lifshitz F, editor. Pediatric Endocrinology. New York, USA: Informa Healthcase; 2009. pp. 531–557.
15. Alonso C, Ureta N, Pallás CR. Vitamina D profiláctica. http://www.aepap.org/previnfad/pdfs/previnfad_vitamina_D.pdf. Consulted 8.1.18.
16. Kelly A, Levine MA. Disorders of Bone and Mineral Metabolism. Pediatric Practice: Endocrinology, 2e Kappy MS, Allen DB, Geffner ME. Kappy MS, Allen DB, Geffner ME. Eds. Michael S. Kappy, et al. New York, NY: McGraw-Hill, 2013, pp. 191-256.
17. CL Wagner, FR Greer. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents Pediatrics, 2008; 122:1142-1152. doi.org/10.1542/peds.2008-1862.
18. Alonso C, Ureta N, Pallás CR. Vitamina D profiláctica http://www.aepap.org/previnfad/pdfs/previnfad_vitamina_D.pdf. Consulted 8.1.18.
19. https://melhorcomsaude.com/12-coisas-sente-deficiencia-de-vitamina-d/. Consulted 29.12.17.
20. Eight Signs and Symptoms of Vitamin D Deficiency. https://www.healthline.com/nutrition/vitamin-d-deficiency-symptoms. Consulted 29.12.17.
21. https://flaviopassos.com/2016/09/11/8-sinais-e-sintomas-de-deficiencia-de-vitamina-d-que-podem-estar-custando-caro-para-sua-saude/ Consulted 29.12.17.
22. Okawa M, Uchiyama M, Ozaki S, and Shirakawa S. Sleep-wake and body temperature rhythms in circadian rhythm sleep-disordes, Japanese-German International Symposium Sleep-Wake Disorders, Oct. 1996, Erfurt, Abtract Book
23. Rosen C, Grossman LS, Harrow M, Bonner-Jackson A, Faull R. Diagnostic and prognostic significance of Schneiderian first-rank symptoms: a 20-year longitudinal study of schizophrenia and bipolar disorder. Compr Psychiatry. 2011; 52(2):126-31. doi: 10.1016/j.comppsych.2010.06.005. Epub 2010 Sep 9.
24. Valoración de los niveles de melatonina. http://www.iimel.es/12-servicios/64-valoracion-de-los-niveles-de-melatonina-2. Consulted 22.2.18.
25. El envejecimiento http://www.iimel.es/el-envejecimiento. Consulted 22.2.18.
26. https://www.jasminealimentos.com/alimentacao/hormonios-da-felicidade-quais-sao-e-o-que-comer-para-ativa-los/. Consulted 9.1.18
27. http://www.paho.org/hq/index.php?option=com_content&view=article&id=13102%3Adepression-lets-talk-says-who-as-depression-tops-list-of-causes-of-ill health & catid =740%3Apress-releases&Itemid=1926&lang=es. Consulted 5.1.18

Effect of DHEA in diabetes type 2 & tuberculosis comorbidity

Manuel Othoniel López Torres[1], Brenda Marquina-Castillo[1], Dulce Adriana Mata[1], Jorge Alberto Barrios-Payan[1], Maria Andrea Carranza[2], Rogelio Hernandez Pando[1]

[1]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[2]Institute of Cardiological Research, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina
lopeztorresmanuel88@gmail.com; brenda.marquina@gmail.com; dulmat@yahoo.com.mx; qcjbp77@yahoo.com.mx; carranza.ma@gmail.com; rhdezpando@hotmail.com

Keywords: cortisol, diabetes type 2, immunotheraphy, tuberculosis (TB), DHEA deficiency

In the last three decades, 314 million new cases of Diabetes Mellitus (DM) were reported. This pandemic disease is a well-recognized risk factor for Tuberculosis (TB) with a 3-fold susceptibility rate increase.

Although studies have found several relations between TB and DM, the specific mechanisms and factors that make diabetic patients more susceptible to developing TB are not yet fully understood. Both diseases are characterized by excessive glucocorticoid production and the overstimulation of 11-β-hydroxysteroid dehydrogenase type 1 (11-βHSD1). This enzyme has reductase activity for 11-ketosteroids products and favors their conversion to cortisol. Steroid hormones, such as dehidroepiandrosterone (DHEA) or its synthetic analog 16-alpha-bromoepiandrosterone (HE2000), which is a modified androstane adrenal hormone without androgenic effects, display immune restoration activity in several infectious and metabolic diseases. Thus, hypercortisolism and decreased DHEA production could contribute to immune suppression in TB-DM individuals. Therefore, we assessed the effect of HE2000 in TB progression in the presence of DM2 in an experimental murine model.

Our results showed that HE2000 diminished bacterial load in lungs in comparison with vehicle control. However, the half-life of the animals was reduced. These results suggest that steroids play a key role in downregulating the immune response and their modulation during comorbid diabetes contributes a reduced immune response that is less effective in controlling bacterial replication within the lungs. The results suggest that HE200 is a potential candidate as an immune adjuvant with standard antibiotic therapy in the disease.

Effect of Caspase-1-dependent inflammation on the protein levels of the BDNF/TRKB and TNF/TNFRII neuroprotector modules in a familial Alzheimer´s disease mouse model

Jorge Luis Ochoa-Almazán[1], Martha Pedraza-Escalona[1], Rodrigo ramos-acevedo[2], Magdalena Guerra-Crespo[2], Leonor Pérez-Martínez[1], Gustavo Pedraza-Alva[1]

[1]*Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico*
[2]*Instituto de Fisiología Celular (IFC), Mexico City, Mexico*
jorge8aal@gmail.com; mapedmx@ibt.unam.mx; rramos@email.ifc.unam.mx; mguerra@ifc.unam.mx; leonor@ibt.unam.mx; gustavo@ibt.unam.mx

Keywords: Alzheimer Disease, Caspase 1, BDNF, Neuroinflammation, TNFR2

Background: Alzheimer's disease (AD) is a neurodegenerative and progressive pathology characterized by memory loss and cognitive deterioration1.

Recently, it has been shown that the inflammatory process that results from the accumulation of β amyloid peptides (βA) and the formation of amyloid plaques plays a crucial role in the neurodegenerative process including the loss of the cognitive and memory capacities2,3. Different research groups, including ours, using mouse models for AD have shown that the inactivation of the inflammasome decreases, in the cortex and hippocampus, the inflammation induced by the βA peptides, the formation of amyloid plaques and improves the cognitive abilities. Therefore, the neuroinflammatory process controlled by the inflammasome plays an essential role in the development of AD4,5. TNF has been implicated in this process6,7; in fact, antibodies that block the binding of TNF to its receptor has been proposed as a promising therapy against the AD8.

Nonetheless, the inflammatory process at early stages of the disease in young mice does not impair learning and memory, indicating that there are mechanisms that can oppose inflammatory signals to maintain neuronal functions. Interestingly, it has been shown that TNF when interacting with the TNFRI promotes neuronal dysfunction and death, however, when it interacts with the TNFRII it promotes survival signals9,10. Thus, alteration in the TNFRI/TNFRII ratio promoted by the presence of βA peptides could also play a role in the outcome of an inflammatory environment on neural function and survival. In addition, BDNF, a neurotrophic factor that exerts neuroprotective and synaptogenic effects, has also been shown to prevent the cognitive deterioration resulting from the accumulation of βA peptides11. Thus, is possible that as long as the

levels of BDNF are maintained, this neurotrophin is able to counteract the deleterious effects of inflammatory cytokines like TNF. However, in postmortem studies, it has been observed that BDNF levels decrease in the cortex and in the hippocampus in late stages of AD12. Likewise, the levels of the truncated form of the TRKB receptor increase, thus impairing BDNF signaling13,14. In contrast, the levels of the immature form of BDNF (pro-BDNF) increases as AD progress15. This, correlates with an increase in the expression levels of p75, the receptor through which pro-BDNF promote neuronal damage15,16. Thus, βA peptides-mediated impaired neuronal function might result from: i) alterations in the ratio BDNF/pro-BDNF; ii) alterations in the ration of TRKB isoforms; iii) increased p75 levels; iv) increased TNF levels; and iv) alteration in the TNFR1/TNFRII ratio. Here, we investigated which of these changes is triggered by caspase-1-dependent inflammation in response to βA accumulation in the 5XFAD murine model for familial AD.

Method: Five and eight months old male wild type (Wt), transgenic 5XFAD, 5xFAD/Caspase-1 KO and Caspase-1 KO (CaspKO) mice in C57/SJL background were used.

In the cortex and hippocampus of these animals, BDNF, pro-BDNF and the receptor TRKB; as well as the levels of the TNF receptors (TNFRI / TNFRII) were evaluated by immunoblot, using specific antibodies.

TNF (50 ng) was administered intracerebroventricularly in 10 weeks old mice. We administered in the left lateral ventricle (anteroposterior: -.22mm, lateral: -1mm and ventral: -2.2mm). 24 hours after TNF icv the novel object test was performed. The test consisted of a training session where the mouse is exposed for five minutes to two equal objects. In the test session, a new object replaced one of the two objects. The time that the mouse explores the new object during five minutes provides an index of recognition memory17. The results obtained were analyzed by one-way ANOVA.

Results: We found that in the cortex and the hippocampus of 5 months old 5xFAD mice, the inflammatory process mediated by caspase-1 activation does not affect the protein levels of BDNF nor the BDNF/proBDNF ratio, compared with the levels found in Wt animals. However, the presence of βA peptide promoted an increase in BDNF levels in the cortex of 8 months old 5xFAD animals that was independent of the inflammatory process mediated by caspase-1, since similar BDNF levels were found in the cortex of 5xFAD-CaspKO mice, compared with the BDNF levels found in Wt animals. Interestingly, the slight increase in proBDNF levels observed in the hippocampus of 5xFAD mice was dependent of caspase-1 activation, since in the hippocampus of 5xFAD-CaspKO mice we found a significant decrease in the proBDNF levels compared

to those found in 5xFAD animals. According to the results described above, the presence of βA peptides did not alters the TNFRI/TNFRII protein ration in 5 months old mice. However, in the hippocampus of 8 months 5xFAD mice we observed a significant decrease in TNFRII protein levels, which was independent of the inflammatory process triggered by caspase-1, since the levels of TNFRII in the hippocampus from 8 months 5xFAD-CaspKO mice also decreased in comparison to the levels observed in WT animals. These data suggest that βA peptide accumulation alter the TNFRI/TNFRII protein levels ratio only in old mice and that this is independent of the inflammatory process triggered by caspase-1.

Together, these results and previous data showing that the inflammatory process induced by the activation of the inflammasome in response to the presence of βA peptides results in cognitive impairment in young mice5, indicate that the negative effect of inflammation on cognitive impairment at early stages of the disease does not involve: i) alterations in the ratio BDNF/pro-BDNF; ii) alterations in TRKB isoforms protein levels; nor iii) alterations in the ratio TNFRI/TNFRII. Thus, pointing out a direct effect of inflammatory cytokines on memory and learning. Accordingly, intraventricular administration of TNF to 10 weeks old mice impaired memory and learning, as determined by the novel objects test. Mice that received TNF expended less time exploring the new object than mice that were injected with the vehicle only. Therefore, elevated TNF levels directly affects the cognitive abilities of young mice despite the presence of BDNF and its receptor. Experiments are underway to discard the possibility that the pro-BDNF-p75 signaling is playing a role in memory impairment resulting from the inflammatory process triggered by inflammasome activation in response to βA peptides.

REFERENCES

1.- Liu,L., & Chan, C. (2014). The role of inflammasome in Alzheimer´s disease. Ageing Research Reviews 0:6-15

2.- G. Zuliani, M. Ranzini, G. Guerra, L. Rossi, M.R. Munari, A. Zurlo, S. Volpato, A.R. Atti, A. Blè, R. Fellin. (2007) Plasma cytokines profile in older subjects with late onset Alzheimer's disease or vascular dementia, J Psychiatr Res 41:686-693.

3.- Lue LF, Rydel R, Brigham EF, Yang LB, Hampel H, Murphy GM Jr, Brachova L, Yan SD, Walker DG, Shen Y, Rogers J. (2001) Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. Glia 35:72-79.

4.- Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Griep A, Axt D, Remus A, Tzeng TC, Gelpi E, Halle A, Korte M, Latz E, Golenbock DT. (2013). NLRP3 is activated in Alzheimer´s disease and contributes to pathology in APP/PS1 mice. Nature, 493(7434), 674–678.

5.- Álvarez-Arellano L, Pedraza-Escalona M, Blanco-Ayala T, Camacho-Concha N, Cortés-Mendoza J, Pérez-Martínez L, Pedraza-Alva G. (2018). Autophagy impairment by caspase-1-dependent inflammation mediates memory loss in response to beta-Amyloid peptide accumulation Journal of Neuroscience Research, 96, 234-246.

6.- Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald KA, Latz E, Moore KJ, Golenbock DT.. (2008). The NALP3 inflammasome is involved in the innate immune response to amyloid-β. Nature Immunology, 9(8), 857–865.

7.- Alvarez A, Cacabelos R, Sanpedro C, García-Fantini M, Aleixandre M. (2007) Serum TNF-alpha levels are increased and correlate negatively with free IGF-I in Alzheimer disease. Neurobiol Aging 28:533-536.

8.- Tobinick EL & Gross H (2008) Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. J Neuroinflammation 5:2.

9.- MacEwan, D. J. (2002). TNF ligands and receptors – a matter of life and death. British Journal of Pharmacology, 135(4), 855–875.

10.- Dong Y, Fischer R, Naudé PJ, Maier O, Nyakas C, Duffey M, Van der Zee EA, Dekens D, Douwenga W, Herrmann A, Guenzi E, Kontermann RE, Pfizenmaier K, Eisel UL.(2016) Essential protective role of tumor necrosis factor receptor 2 in neurodegeneration. Proc Natl Acad Sci U S A. 113(43):12304-12309

11.- Nagahara AH, Merrill DA, Coppola G, Tsukada S, Schroeder BE, Shaked GM, Wang L, Blesch A, Kim A, Conner JM, Rockenstein E, Chao MV, Koo EH, Geschwind D, Masliah E, Chiba AA, Tuszynski MH.. (2009) Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. Nat Med 15:331-337.

12.- Connor B, Young D, Yan Q, Faull RL, Synek B, Dragunow M.. (1997) Brain-derived neurotrophic factor is reduced in Alzheimer's disease. Brain Res Mol Brain Res 49:71-81.

13.- Connor B, Young D, Lawlor P, Gai W, Waldvogel H, Faull RL, Dragunow M. (1996) Trk receptor alterations in Alzheimer's disease. Brain Res Mol Brain Res. 42(1):1-17

14.- Ferrer I, Marín C, Rey MJ, Ribalta T, Goutan E, Blanco R, Tolosa E, Martí E. (1999) BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. J Neuropathol Exp Neurol. 58(7):729-39.

15.- Jung Yeon Lim,1 Charles P. Reighard,2 and Damian C. Crowther.(2015) The pro-domains of neurotrophins, including BDNF, are linked to Alzheimer's disease through a toxic synergy with Aβ. Hum Mol Genet. 24(14): 3929–3938.

16.- Chakravarthy B, Ménard M, Ito S, Gaudet C, Dal Prà I, Armato U, Whitfield J. (2012) Hippocampal membrane-associated p75NTR levels are increased in Alzheimer's disease. J Alzheimers Dis. 30(3):675-84.

17.- Leger M, Quiedeville A, Bouet V, Haelewyn B, Boulouard M, Schumann-Bard P, Freret T. (2013) Object recognition test in mice. Nat Protoc. 8(12):2531-7.

Development of human monoclonal antibodies against multidrug resistant bacteria

Sugey Bravo[1], Marbella Ovilla-Muñoz[1], Sara Bahena[1], Andrea Avilés[1], Lina Restrepo[2], Juan Téllez-Sosa[1], Jesus Martinez-Barnetche[1]

[1]*Instituto Nacional de Salud Pública, Cuernavaca, Mexico*
[2]*EAFIT University, Medellín, Colombia*
flowersusuki@gmail.com; movilla@insp.mx; Yuvisel_71@yahoo.com; andy_sa@live.com.mx; lrestr77@eafit.edu.co; jmtellez@insp.mx; jmbarnet@insp.mx

Keywords: Staphylococcus aureus, monoclonal antibodies, multidrug resistant bacteria, Enterobacter sp., Klebsiella pneumoniae (K. pneumoniae)

Introduction: According to the first report published by the WHO in 2014, the multi-resistant bacteria Staphylococcus aureus, Enterobacter sp., Klebsiella pneumoniae and Acinetobacter baumanni, represent a worldwide menace, because they do not respond to ordinary treatment, giving rise to prolonged diseases, increase the risk of death and elevated medical treatment costs. Due to the ineffectiveness of antibiotics and the lack of current vaccines to treat this type of infections, monoclonal antibodies (mAbs) represent an alternative for the treatment of infections generated by multi-resistant bacteria.

Objetive: To identify human mAbs with therapeutic potential for the treatment of infections caused by multi-resistant bacteria.

Method: Purified peripheral blood B cells, from buffy coat donated by the Blood Bank of the State of Morelos, were stimulated in vitro in the presence of antigens and a mix of polyclonal activators (CpG, IL- 21 and anti-CD40) in conditions that favor antigen-specific clonal selection. As antigen, specific targets were used (recombinant external membrane proteins from E. cloacae, K. pneumoniae and A. baumannii selected by a bioinformatic analysis); or fixed cells (SpA-null S. aureus). On the sixth day post-stimulation, B cells were used to identify antigen-specific cells by ELISPOT assays. The RNA of the B cells secreting antibodies of interest was used to amplify, by 5' RACE- PCR, libraries of the variable regions of IgG and IgK. The libraries were massively sequenced (MiSeq) and their bioinformatic analysis guided the selection of VDJ rearrangements of heavy and light chains. The selected variable regions were used to generate recombinant mAbs in HEK293T cells. The specificity and functionality of such antibodies will be validated in vitro and in vivo assays.

Results: Five recombinant outer membrane proteins, highly conserved in E. cloacae and K. pneumoniae, were used as antigens for in vitro B cells activation and ELISPOT assays to identify cells that produce antigen-specific antibodies. Recombinant mAbs generated against 4 of these proteins are currently subjected to specificity assays. In the case of A. baumannii, 5 highly conserved outer membrane proteins were selected and are currently being recombinantly produced. Finally, in the case of S. aureus, we standardized the in vitro activation of B cells and detected, by ELISPOT assays, cells that produce specific IgG antibodies against outer membrane components other than the SpA protein.

Recombinant domains I/II and III of dengue virus- 2 and humoral immune response in patients with dengue severe and no severe of endemic area

Josselin Carolina Corzo[1], Alfredo Eduardo Montes[1], Maria Gonzalez[2], Julio García[1], Mussaret Zaidi[2], Leopoldo Santos-Argumedo[1], Leticia Cedillo-Barron[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
[2]Hospital General O'Horan, Merida, Mexico
josselin.corzo@hotmail.com; freddym14@hotmail.com; maria.gonzalez@ssy.gob.mx; lebeeydengue@yahoo.com.mx; mussaret_zaidi@yahoo.com; lesantos@cinvestav.mx; lcedillo@cinvestav.mx

Keywords: Dengue, recombinant protein, human serum, endemic area, EDIII, EDI/II

Dengue (DEN) is a disease caused by four serologically and genetically related viruses, termed DENV types 1–4, which belong to the genus Flavivirus, family Flaviviridae. Dengue is the most important mosquito-borne viral disease in terms of the number of cases each year and its geographic distribution (Messina et al., 2014), this infection is highly prevalent in tropical and subtropical areas. Specifically dengue remains a public health problem in the Americas. In Mexico, the number of dengue cases in the last year were 1,793 of which 477 were severe cases (PAHO 2017). Currently there is no licensed vaccine or specific treatment. DENV is a single-stranded, positive-sense RNA virus with a genome of approximately 11 kb. It´s genome contains a single open reading frame encoding three structural proteins: capsid (C), pre-membrane/membrane (prM/M), and the envelope glycoprotein (E) and seven nonstructural proteins (Rice et al., 1985). The DENV E protein is a major determinant of tropism and the primary target of virus-neutralizing antibody. This protein has three domains; Domain I is a central β- barrel , Domain II is an elongated dimerization region and a C-terminal immunoglobulin (Ig)-like module (Modis et al., 2003), which is important to cellular receptor binding. Most patients infected with dengue recover following a self-limiting non severe clinical disease but a small proportion progress to severe disease, mostly characterized by plasma leakage with or without hemorrhage, this leading cause of hospitalization and death among children and adults (WHO 2009). Recovery from infection by one serotype provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary, but subsequent infections by other serotypes, increase the risk of developing severe dengue.

The human samples were collected in collaboration with the O'Horán General Hospital of Mérida, Yucatán, under the bioethical guidelines established for human research, all individuals who agree to participate will grant their informed consent letter to obtain samples blood. The patients must meet the following inclusion criteria:

- Resident patients of Yucatan, of any age that come to consultation.
- Present or have presented in the last 7 days, acute onset fever (≥38 ° C) for at least two continuous days accompanied by one or more of the following symptoms; headache, myalgia, arthralgia, rash, retro-orbital pain.

DI/DII from E protein sequences were cloned into the pMTBvector (expression plasmid of Drosophila melanogaster (Invitrogen, Carlsbad, CA, USA)), which is under the control of the metallothionein promoter and nducible by CuSO4. In the other hand, DIII from E protein sequence were cloned into the in pPROEX HT, this plasmid containing the inducible lac promoter and it's inducible by isopropyl- β -D-1 thiogalactopyranoside (IPTG). Their sequences were amplified by reverse transcription (RT)-PCR from DENV-2 infected Aedes albopictus C6/36 cells using appropriate oligonucleotides. PCR products were digested with the appropriate restriction enzymes and inserted into the different plasmids. The proteins were purified by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously by Katrak et al. (1992). The proteins were then eluted from the macerated gel bands with phosphate-buffered saline (PBS) and their protein concentrations were determined (Bio-Rad, Hercules, CA).

For antibody detection using ELISA; serum of individuals were tested against recombinant protein EDI/DII and EDIII. These purified proteins were coated using 96-well polyvinyl plates (Nunc) at 3μg/ml in carbonate/bicarbonate buffer (pH 9.6); the plates were incubated overnight at 4°C. Wells were blocked with 0.05% (v/v) Tween 20 and 3% (w/v) bovine serum albumin in PBS and then three serial dilutions of test sera were incubated for 1 hour at 37°C. Bound specific antisera were detected using a secondary antibody goat peroxidase-conjugated anti-human IgM and IgG (ABCAM) respectively, followed by the addition of substrate. Absorbance values were determined at 450 nm. The cutoff OD for testing the seropositivity of each sample was defined as an adjusted OD the mean plus 2 standard deviations of the negative control sera. Negative and positive control sera were included in each assay.

The aim of this study is analyzed antibody profiles in patients with classical and severe dengue from southeast of Mexico and its correlation between antibody levels and clinical differences. Firstly, people in febrile stage sought help and were treated in the hospital O´Horan of Merida, Yucatan, Mexico. All participants were screened for the presence of DENV IgM and IgG antibody levels and eighty of these were diagnosed with

dengue fever and classified according to the criteria established in the 2009 WHO guide. Out of the total dengue cases 65.7% was classic dengue and 32.5% was detected with severe dengue. After that we used recombinants proteins EDI/DII and EDIII obtained in Drosophila S2 and E. coli strain C-43, expression system, respectively and antibody levels were determinate. The demographic data among the patients infected with dengue was 31.3% (25/80) were found in the age group of 0–19 years, 37.5% (30/80) were 20–59 years and 18.75% (15/80) were older than 60 years old, respectively. Of these, 50% were male and other 50% were female. The mean age of the subjects was 29.03 years with the youngest and the oldest age being 1 and 98 respectively. We compared between patients infected with dengue (cases), healthy volunteers residents of Yucatan (negative controls of endemic area) and healthy volunteers of Zacatecas (negative controls of no endemic area) and we can observe that IgG levels was significantly higher against protein EDI/DII in non-severe dengue patients compared to severe dengue ones and there are similar to the levels observed in healthy individuals from the same endemic area, meanwhile no antibody response was detected in individuals of non-endemic area. In the other hand, the antibody response against EDIII was significantly lower than antibody response against EDI/DII in the same patients with dengue.

This information suggests that the domain EDI/DII is the most immunodominant in E protein and that the antibody response is higher in people with classic dengue. Probably these high levels of antibodies arrive at critical threshold that facilitates the prevention of disease progression but is necessary to do neutralization assay for to prove it.

REFERENCES

- Messina et al. Global spread of dengue virus types: mapping the 70 year history.Trends in Microbiology 2014; Vol. 22 No.3.
- http://www.who.int/denguecontrol/epidemiology/en/
- Modis, Y., et al., A ligand-binding pocket in the dengue virus envelope glycoprotein. Proc Natl Acad Sci U S A, 2003. 100(12): p. 6986-91.
- Halstead, S.B., Dengue hemorrhagic fever. Trans R Soc Trop Med Hyg, 1988. 77(5): p. 739-40.
- World Health Organization., Dengue guidelines for diagnosis, treatment, prevention and control: new edition. 2009, Geneva: World Health Organization. X, 147 p.
- Katrak, K., et al., Preparative separation of foreign antigens for highly efficient presentation to T cells in vitro. J Immunol Methods 1992; 156:247–254.

Molecular analysis of NKT cells from mice with Lupus induced by the stabilization of non-bilayer phospholipid arrangements

Carlos Wong Baeza[1], Albany Reséndiz-Mora[1], Carla Landa[1], Mitzi Camacho[1], Elizabeth Martínez-Rostro[1], Carlos Wong[1], Leopoldo Flores-Romo[2], Isabel Wong-Baeza[1], Isabel Baeza[1]

[1]Instituto Politécnico Nacional, Mexico City, Mexico
[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
charlywong@icloud.com; cbanyrm@icloud.com; carla_tao@hotmail.com; qbp.clms@gmail.com; lissiee@icloud.com; carloswongr@yahoo.com; leflores@cinvestav.mx; isa_wb@yahoo.com.mx; isabelbaeza@yahoo.com

Keywords: Gene Expression, NKT cells, systemic lupus erythematosus, non-bilayer phospholipid arrangements, anti-lipid IgG antibodies

In an immune response against protein antigens, follicular T cells cooperate with B cells; as a result of this cooperation, B cells react via germinal center, where they switch their antibody isotype and mutate the genes that encode their antigen receptors. These processes can change the antibody affinity and even the antibody specificity. The mutated cells that produce high-affinity antibodies are selected to become either plasma cells or memory B cells, while cells that have lost affinity or acquired auto-reactivity are typically eliminated. On the other hand, in an immune response against lipid antigens, B cells can not cooperate with follicular T cells, because these cells are specific for protein antigens, but they can cooperate with NKT cells that have receptors that recognize lipid antigens. B cells then react via germinal center to produce high affinity antibodies against lipids. However, the ability of NKT cells to initiate germinal center reactions is not clear, because this ability has only been partially studied with alpha-galactosylceramide, a glycolipid from marine sponges that is used to activate these cells. Non-bilayer phospholipid arrangements (NPA) are transient, non-immunogenic lipid associations different from the bilayer, but if they are stabilized by some drugs (such as chlorpromazine) they become immunogenic and induce anti-NPA antibodies. These antibodies are found in patients with systemic lupus erythematosus (SLE) and trigger a lupus-like disease in mice. Mice with this disease have anti-nuclear, anti-histone, anti-coagulant and anti-cardiolipin antibodies, weight loss, glomerulonephritis, anorexia and facial lesions resembling the malar rash of the patients. In our research group, we have shown that, in the model of Lupus generated by the stabilization of NPA in mice, B cells mainly react via germinal center to produce high affinity IgG antibodies against NPA (a lipid antigen). Accordingly, in this study we

found an increased number of NKT cells in the draining lymph node and in the spleen of the mice with lupus, and that these cells are activated and produce IL-4. Therefore, we separated the NKT cells by flow cytometry and evaluated the expression of genes related with the innate and adaptive immune responses. The NKT cells over-expressed Foxp3, Irf-7, Tlr9, Mx1 and IFNγ compared with the mice administrated with saline solution. Our results suggest the involvement of NKT cells in the adaptive immune response that leads to the production of high affinity anti-NPA IgG antibodies and to the development of the Lupus-like disease in mice.

IgG, but not IgA, protects infant mice against oral C. rodentium infection

Gustavo Caballero Flores, Melody Zeng, Naohiro Inohara, Gabriel Núñez

University of Michigan, Ann Arbor, United States
gcflores@med.umich.edu; mzeng@med.umich.edu; nao@med.umich.edu; bclx@med.umich.edu

Keywords: IgA, IgG, enteric pathogens, Diarrheal diseases, C. rodentium

Enteric and diarrheal diseases are the second cause of death in children under 5 years, causing near 1 million deaths per year worldwide. The increased susceptibility of neonates to enteric pathogens is due in part to the immaturity of their immune system. In this study, we tested the role of IgG in protecting infant mice against C. rodentium, a mouse-specific pathogen that models infection by enteropathogenic Escherichia coli (EPEC), one of the most common cause of diarrheal deaths in neonates worldwide. We found that antibodies reactive against C. rodentium reduced pathogen loads, mortality and intestinal pathology in neonates challenged with the pathogen. We also observed that protection was abolished by deficiency of FcRn, the receptor that mediates the transfer of IgG from serum to the intestinal lumen in neonates, but not by the absence of IgA or IgM, thus indicating that IgG, but not IgA or IgM, plays a critical role in neonatal protection against C. rodentium infection. To determine the in vivo importance of delivery of IgG directly to the intestine of infant mice, naive pups were cross-fostered by mothers showing antibodies reactive to the pathogen, while pups derived from such moms were nursed by naive females. We observed reduced pathogen loads and mortality in the naive pups nursed by infected moms, suggesting that mucosal IgG plays a critical role in conferring protection to the neonate against infection. Our results indicate that delivery of IgG to the intestine protects neonates from oral infection with C. rodentium. This study may pave the way to the development of effective strategies to prevent disease and death caused by enteric pathogens in the neonatal population.

This work was partially supported by a Postdoctoral Fellowship from National Council on Science and Technology (CONACYT, #232554).

Evaluation of the antiangiogenic effect of vNAR antibodies against VEGF isolated from synthetic antibody libraries

Olivia Cabanillas-Bernal[1], Teresa Escalante[2], Alexei Fedorovish Licea Navarro[1]

[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico
[2]University of Costa Rica, San José, Costa Rica
vivi_057@hotmail.com; tescalan3000@gmail.com; alicea@cicese.mx

Keywords: Antibodies, VEGF, Angiogenesis, vNAR, Synthetic libraries

Introduction: The single domain shark antibodies IgNAR, present special characteristic over conventional antibody molecules such as a small size (12–15 kDa), a long and extended CDR3, better tissue penetration, thermic and chemical stability, resistance to gastric pH, among others. These characteristic make them ideal candidates for biotechnological and therapeutic uses. Recently, efforts have been made to try to avoid the limitations of developing antibodies in animals, through the use of techniques that are completely in vitro for the design of antibodies with adapted specificity. This has been possible with the advent of synthetic antibody libraries, that have diversity outside the scope of application of the natural immune repertories. An important advantage of the synthetic libraries is that they allow the relatively fast and easy generation of recombinant antibodies with specificity for practically any antigen of interest.

Objective: Isolate antibodies from a synthetic library capable of neutralizing the vascular endothelial growth factor (VEGF) and inhibit the angiogenesis in vitro.

Methods: In this study, two antibodies were rapidly and easily selected (NAR04 and NAR120) against the VEGF. The antibodies were selected from three synthetic libraries of vNAR antibodies, utilizing phage display technology, during four rounds of selection. The cognate antigen recognition ability of the selected antibodies was evaluated by recognition ELISA. Additionally, the antiangiogenic effect of the antibodies was evaluated in a three dimensional spheroid angiogenesis model of endothelial cells.

Results: The two antibodies were successfully expressed as a soluble protein in the periplasm of Escherichia coli. Both proteins presented a size of approximately 14 kDa when immunodetected by wester blot. The expression levels of both recombinant

proteins were approximately 0.2 mg/mL. Both antibodies showed the ability to recognize and neutralize VEGF in vitro.

Conclusions: Our results suggest that the use of synthetic antibody libraries provide a fast and easy way to isolate antibodies as we have shown against VEGF. These synthetic antibodies showed the capability to neutralize their antigen and inhibit angiogenesis in vitro. The use of synthetic antibodies could rival natural immune antibodies, avoiding animal immunization and shortening the production time needed to develop an antibody.

CEA labeling on the surface of colon cancer cells, employing a shark vNAR antibody

Olivia Cabanillas-Bernal, Luis Donis-Maturano, Alexei Fedorovish Licea Navarro

Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico
vivi_057@hotmail.com; ludoma6@hotmail.com; alicea@cicese.mx

Keywords: Antibodies, Flow Cytometry, colorectal cancer, CEA, vNAR

Introduction: The carcinoembronegic antigen (CEA) is a glycoprotein that is overexpressed in various types of cancer and it is widely used as an important clinic tumor marker for colorectal carcinomas and other carcinomas. Various studies have employed monoclonal human or murine antibodies (mAbs) to localize CEA in solid tumor, however due to their size the mAbs have a limited penetration in solid tumors.

Due to their special characteristics such as a small size (12–15 kDa), long and extended CDR3, better tissue penetration, thermic and chemical stability, resistance to gastric pH, among others, the vNAR shark antibodies represent a potential alternative to conventional antibodies. Therefore, vNARs can be employed as a detection method for molecules of therapeutic interest, such as CEA.

Objective: Isolate a vNAR shark antibody capable of recognizing CEA on the surface of cancer colon cells.

Methods: A selection of antibodies against CEA was carried out from a synthetic vNAR library, utilizing phage display technology. The ability of the selected antibodies to recognize soluble CEA was evaluated by a recognition ELISA, and the ability to recognize CEA on the surface of colon cancer cells HCT-116 and breast cancer cells MDA-231 (negative control) was evaluated by flow cytometry.

Results: A vNAR anti-CEA antibody was isolated. The antibody was successfully expressed as a soluble protein using Escherichia coli as an expression model and was capable of recognizing soluble CEA employing a recognition ELISA. The evaluation of the extracellular labeling of CEA with the vNAR antibody in the HCT-116 and

MDA-231 cell lines by flow cytometry showed an increase of the mean fluorescence intensity (MFI) only in the colon cancer cells HTC-116 that expressed CEA in a constitutive manner.

Conclusion: A vNAR antibody was isolated against CEA from a synthetic antibody library, capable of recognizing soluble CEA and extracellular CEA on the surface of colon cancer cells. Due to their small size (14 kDa) this antibody represents a potential option as an agent to detect CEA in the serum and solid tumors.

Pathogenicity of auto-antibodies from man to mice: the case of immune-mediated necrotizing myopathies

Olivier Boyer[1], Cécile Bergua[1], Olivier Benveniste[2], Laurent Drouot[1]

[1]Normandy University, Rouen, France
[2]Sorbonne Universités, Paris, France
olivier.boyer@chu-rouen.fr; cecile.bergua44@gmail.com; olivier.benveniste@aphp.fr; laurent.drouot@univ-rouen.fr

Immune-mediated necrotizing myopathies (IMNM) are a newly recognized group of severe acquired myopathies associated to auto-antibodies (aAbs) directed against the signal recognition particle (SRP) or 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR). Besides their usefulness as diagnostic biomarkers, it remains to determine whether IMNM-associated are simple biomarkers or directly pathogenic.

Keywords: Autoantibodies, Autoimmune Diseases, Immune-mediated necrotizing myopathy, Anti-HMGCR, anti-SRP

Methods: Recombinant human SRP or HMGCR was used to measure aAb levels by ALBIA. Immunostaining and reverse transcription PCR were performed on muscle biopsies and primary muscle cell cultures. Atrophy and regeneration were evaluated based on the myotube surface area as well as gene and cytokine profiles, after incubation with aAbs. IgGs from patients were transferred to normal, Rag2-/- or complement C3-/- mice, supplemented or not with human complement. Muscle strength was evaluated by grip test and in situ muscle contraction upon sciatic nerve electrostimulation.

Results: aAb titers correlated with CK levels and/or disease severity. SRP and HMGCR were detected on altered myofibers and on myotubes. Sarcolemmal complement deposits correlated with myofiber necrosis. In vitro, anti-SRP and anti-HMGCR Abs provoked myotube atrophy, and increased transcription of atrophy-related genes, inflammatory cytokines and reactive oxygen species. aAbs impaired myoblast fusion and muscle regeneration. In vivo, patients IgG decreased muscle strength transiently or permanently in immunocompetent or immunodeficient mice, respectively. Pathogenicity was reduced in C3-/- mice while increased by human complement. Immunization with SRP or HMGCR provoked a muscle deficit.

Conclusion: Rather than being simple biomarkers of disease, anti-SRP and anti-HMGCR aAbs are true pathogenic effectors that provoke atrophy, impaired regeneration and necrosis through a complement-dependent mechanism, prompting to evaluate complement- and B-cell targeting therapies in IMNM.

Myosin Ie supports the migration of B cells

Daniel Alberto Giron Perez, Eduardo Vadillo, Michael Schnoor, Leopoldo Santos-Argumedo

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
daniel_2555@outlook.com; evadillo@hotmail.com; mschnoor@cinvestav.mx; lesanto@cinvestav.mx

Keywords: Migration, B cells, Adhesion, intravital microscopy, Myosin Ie

Introduction: Mature B cells move through the lymph nodes to encounter antigens and to generate antibodies. They leave the vascular system and enter lymph nodes via high endothelial venules (HEV). The lymph node is divided in paracortical, cortical and medullar zones; and the migration of B cells in the cortical zone is mediated by a gradient of the chemokine CXCL12 released by follicular dendritic cells (FDC). B-cells modulate their cell shape for migration, phagocytosis and during antigen uptake; which requires dynamic actin cytoskeleton remodeling mediated by myosin motor proteins. There are 18 members of the myosin family with different functions. Among these, class I myosins contain only a monomeric heavy chain with three regions (motor, neck, and tail) each serving specific cellular functions.

Myosin-1e is encoded by chromosome 9 in mice and 15 in humans and contains 1108 aminoacids. B-cells express high amounts of Myo1e. In silico analysis revealed an association of Myo1e with the actin nucleation promotion factor WASp, ARP2/3 and Coronins, which are known to regulate B-cell migration. However, the direct role of myosin-1e in B cell migration has yet not been explored.

Aim: Previously, our group reported the expression of three class I myosins in B-cells (Myo Ic, Myo Ig and, Myo Ie). To analyze B-cell migration in vivo in Myo1e-deficient mice by intravital microscopy.

Results: Hoechst-labeled Myo1e-/- and wild-type (WT) B-cells were used. B-cell migration was recorded by intravital microscopy of inguinal lymph nodes 2 hours after injection recipient C57BL6J mice with CXCL12 (50 ng/ml) local stimulation. To analyze the motility of Myo1e-/- and WT B cells, 1-hour real-time videos from venules of inguinal lymph nodes were taken, and cell flux, rolling, adhesion and trans-migration were analyzed. All parameters were altered in the MyoIe-/- compared with WT B cells. Lateral migration in a chemokine gradient (CXCL12), was evaluated in

1-hour overlay of images. Analysis of tracks from individual cells showed a reduction in speed, accumulated and euclidian distance in Myo1e-/-. Confocal microscopy showed co-localization of CXCR4 with Myosin1e at the pseudopod.

To migrate, cells must first adhere to the substrates using surface adhesion molecules such as CD44, VLA-4 and, LFA-1. These molecules have high affinity to different ligands and mediate the adhesion of B-cells in the endothelium of inguinal lymph nodes. Thus, expression of these proteins on the surface is important for the interaction of B cells with their substrates. Adhesion experiments using MyoIe-/- and WT B cells with different substrates (poly-L-lysine, fibronectin and hyaluronic acid) showed reduced adhesion rates in the absence of myosin1e. Moreover, Myo1e-/- B-cells showed reduction surface expression of CD44, VLA-4 and, LFA-1. However, total expression was not affected. These results imply that Myo1e deficiency may cause internalization of these molecules.

CARMIL is a multidomain protein with actin-related functions. It may serve as a scaffold for the ARP2/3 complex and myosin-1e. CARMIL is involved in the regulation of actin filaments to generate lamellipodia at the pseudopod. Confocal microscopy analysis showed reduced localization of CARMIL at the border leader of MyoIe-/- B-cells.

Conclusion: Myo1e deficiency causes dysregulation in the migration of B cells in inguinal lymph nodes. It is possible that this molecule interacts with other NPF proteins and/or GTPases. Thus, more studies are needed to understand its mechanism of action.

Understanding Class Switch Recombination related non-coding (CSR-nc) transcription through experimental analysis and data mining of RNA-seq experiments at the NCBI Sequence Read Archive, the GTEx and TCGA projects

Helena Kuri-Magaña[1,2], Leonardo Collado-Torres[3], Juan Téllez-Sosa[2], Marbella Ovilla-Muñoz[2], Humberto Valdovinos-Torres[2], Laura Bonifaz[4], Jesus Martinez-Barnetche[2]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Nacional de Salud Pública, Cuernavaca, Mexico
[3]Lieber Institute for Brain Development, Baltimore, United States
[4]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
helenakuri@gmail.com; leo.collado@libd.org; jmtellez@insp.mx; movilla@insp.mx; htorres@insp.mx; labonifaz@yahoo.com; jmbarnet@insp.mx

Keywords: noncoding RNA, B cells, activation-induced cytidine deaminase (AID), Class switch recombination, IgH locus, RNA-seq analysis

The hallmark of the humoral adaptive immune response is the production of high affinity class-switched antibodies. Affinity maturation and class switch recombination (CSR) are highly regulated, T cell-dependent mutational processes that occur in a specialized microenvironment within secondary lymphoid organs known as the germinal center (GC). Antibody class switching to IgG, IgA or IgE allows effector function diversification beyond IgM. Upon antigen stimulation, IgM+ B cells undergo proliferation in the GC and initiate CSR, which involves a genomic deletion of the IgM/IgD coding interval to the upstream flank of the new recombining class. The initiation of CSR depends on non-coding transcription of i-exons 5' of each S and IGH coding constant region genes (Yewdell and Chaudhuri, 2017). Although the mechanistic knowledge regarding CSR has advanced greatly in the mouse model, our knowledge about ncRNAs involved in CSR in health and disease in humans is limited.

CSR-nc transcription is coupled to chromatin remodeling, RNApol II-mediated transcription of i-exons, R-loop formation and recruitment of Activation Induced Cytidine Deaminase (AID). Cytidine deamination in S regions activates DNA damage response, resulting in a double-strand break in both donor and acceptor S regions, the deletion of the intervening region, as well as the S-S synapsis within the acceptor IGHC region gene.

We hypothesized that CSR-nc transcription signals a distinctive event (i.e., the stage when CSR is actually occurring as a result of T-dependent antigen stimulation) beyond the transcription of switched coding transcripts (i.e. resting memory B cells), and that its quantitation could lead to further understanding of the normal and pathological immune response. To characterize these CSR-ncRNA, we analyzed more than 70,000 publicly available RNA-seq runs from a wide variety of research projects, including the Genotype Tissue Expression project (GTEx. https://www.gtexportal.org/home/), The Cancer Genome Atlas (TCGA. https://cancergenome.nih.gov/) and more than 2,000 projects from the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) using recount2 (Collado-Torres et al., 2017), an R language based tool coupled to an online resource consisting of normalized RNA-seq gene and exon counts, as well as coverage bigwig files (https://jhubiostatistics.shinyapps.io/recount/).

We first selected SRA database projects containing samples from normal isolated B cells to map CSR-nc transcription. S regions were also mapped by determining ATGC motif frequency in 500 bp windows across the IGH locus and visualized with Interactive Genome Viewer (IGV). The corresponding bigwig files were loaded into to IGV to identify visually the coverage distribution regarding the position of IGHC genes and their corresponding S region. This allowed us to clearly map i-exon transcription for the human IGHC genes, including the poorly understood IGHGD gene. To verify the transcription of putative i-exon transcription, we developed a qRT-PCR assay for ncRNA IgM, IgG1 and IgG3 and noted an increase of CSR-nc transcription 7 days post-vaccination with Influenza, Hepatitis B or Tetanus-Diphtheria vaccines.

We then queried the complete recount2 database to obtain normalized expression data (Reads Per Kilobase Million, RPKM) for each i-exon. Overall, the main i-exon transcribed was for i-E . We used GTEx runs as a reference for normal tissue and we found the presence of these transcripts mainly in blood, spleen, lung, gastro-intestinal tract, genito-urinary tract, breast, salivary glands and omentum, as well we found the absence of transcripts in tissues such as brain, skin, muscle and heart. Of note, some anatomical terms such as testis, thyroid and pituitary gland are enriched in CSR-ncRNA expressing runs, despite they are considered immune-privileged sites and are devoid of secondary/tertiary lymphoid organs (Chen et al., 2016). CSR-nc transcription in omentum, namely i-E 1 and i-E 4 highlights the role of adipose lymphoid B cells as mature immunologically active cells having a homeostatic role, but may also play important roles in metabolic syndrome and obesity (Winer et al., 2014).

The TCGA project was used as a reference to study active CSR in a wide variety of human cancers. As expected, high CSR-nc transcription was found in chronic lymphoid leukemia and diffuse large B cell lymphoma, but also in acute myelogenous leukemia. In general, CSR-nc expression in cancer tissues mimicked the expression

of their normal counterparts, with the exception of hepatocellular carcinoma. In at least lung, breast and colorectal carcinomas, the source of CSR-ncRNA appears to be the mucosal associated lymphoid tissues and tertiary lymphoid infiltrates resulting from tumor-associated inflammation (Colbeck et al., 2017), since CSR-ncRNA was not significantly detected in their corresponding tumor-derived immortal cell lines such as A549, MCF-7, and HCT116, respectively.

The SRA dataset represents the most diverse collection of data regarding methodological approaches and subjects of interest. Analysis of the SRA allowed us to cross-validate our findings derived from the GTEx and TCGA projects with matched independent experiments. It also complemented the analysis of some normal tissues not included in the GTEx project, such as bone marrow in which CSR-nc transcription is absent. It also provided a robust dataset of EBV-immortalized lymphoblastoid cell lines form different ethnical origins, and greatly increased the type of B cell malignancies which presented particular patterns of CSR-nc expression. Moreover, the SRA allowed us to identify enriched CSR-nc expression in infectious diseases such as diarrhea, brucellosis and malaria, autoimmune disease such as systemic lupus erythematous, an a particular pattern with increased i-E transcription in Crohn's disease, suggesting a role of IgD+ B cells in regulating mucosal inflammation (Chen and Cerutti, 2011). SRA data from influenza vaccination was consistent with our own experimental data demonstrating an increase of CSR-nc transcription 7 days post-vaccination coinciding with the plasmablast migration wave to peripheral blood, suggesting that CSR-nc transcripts derive from recently class-switched plasmablast, highlighting the use of CSR-nc transcripts as markers of GC responses.

In conclusion, CSR-nc transcription occurs in multiple anatomical locations beyond classical secondary lymphoid organs, representing a useful marker of effector T-dependent B cell responses in normal immune responses (i.e. vaccination), as well as in pathological immune responses. The pattern of i-exon expression may reveal clues of the local immune response in health and disease. The interaction between particular microenvironments (i.e. cytokine milieu) in determining particular patterns of CSR ex vivo is currently under investigation.

REFERENCES

Colbeck, E.J., Ager, A., Gallimore, A., and Jones, G.W. (2017). Tertiary Lymphoid Structures in Cancer: Drivers of Antitumor Immunity, Immunosuppression, or Bystander Sentinels in Disease? Front Immunol 8, 1830. doi: 10.3389/fimmu.2017.01830.

Collado-Torres, L., Nellore, A., Kammers, K., Ellis, S.E., Taub, M.A., Hansen, K.D., et al. (2017). Reproducible RNA-seq analysis using recount2. Nat Biotechnol 35(4), 319-321. doi: 10.1038/nbt.3838.

Chen, K., and Cerutti, A. (2011). The function and regulation of immunoglobulin D. Curr Opin Immunol 23(3), 345-352. doi: 10.1016/j.coi.2011.01.006.

Chen, Q., Deng, T., and Han, D. (2016). Testicular immunoregulation and spermatogenesis. Semin Cell Dev Biol 59, 157-165. doi: 10.1016/j.semcdb.2016.01.019.

Winer, D.A., Winer, S., Chng, M.H., Shen, L., and Engleman, E.G. (2014). B Lymphocytes in obesity-related adipose tissue inflammation and insulin resistance. Cell Mol Life Sci 71(6), 1033-1043. doi: 10.1007/s00018-013-1486-y.

Yewdell, W.T., and Chaudhuri, J. (2017). A transcriptional serenAID: the role of noncoding RNAs in class switch recombination. Int Immunol 29(4), 183-196. doi: 10.1093/intimm/dxx027.

Cellular and molecular characterization of human memory B cell response upon dengue virus infection and other arboviruses

Mireille Aline Santamaria[1,2], Juan Téllez-Sosa[2], Laura Bonifaz[3], Veronica Andrade[4], Jesus Martinez-Barnetche[2]

[1]National Autonomous University of Mexico, Cuernavaca, Mexico
[2]Instituto Nacional de Salud Pública, Cuernavaca, Mexico
[3]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
[4]Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE), Mexico City, Mexico
msantamaria@comunidad.unam.mx; jmtellez@insp.mx; labonifaz@yahoo.com; veroandrade_72@hotmail.com; jmbarnet@insp.mx

Keywords: Arboviruses, Dengue Virus, Somatic Hypermutation, Immunoglobulin, Antibody repertoire, memory B cell

Dengue is the most common vector borne viral disease worldwide, with approximately 400 million cases per year, and a variable clinical spectra ranging from asymptomatic infection to severe life threatening disease. Epidemiological data indicates an elevated risk of severe disease in secondary infections (Bhatt et al., 2013). Population based-serological studies, as well as experimental data suggest a possible role of preexisting non-neutralizing heterotypic antibodies in potentiating severe disease, among other non-mutually exclusive pathogenic mechanisms (Guzman and Harris, 2015; Katzelnick et al., 2017). The efficacy of Dengevaxia®, currently the only licensed vaccine is far from ideal and highly controversial due to safety issues. Thus, better understanding of the anti-Dengue immune response is critical for the development and evaluation of novel and ongoing vaccination strategies. We have previously shown lower somatic hypermutation rates and preferential IGHV usage in the IgG compartment of peripheral B cells during acute Dengue virus infection, regardless if it was a primary or a secondary infection, suggesting that Dengue infection is accompanied by a robust germinal center-independent (GC-i) response involving class switching but little SHM (Godoy-Lozano et al., 2016). To further understand the B cell subpopulation kinetics and clonal relationships, we have extended our analysis in human infection with Dengue or Zika virus, an antigenically related flavivirus. We measured the plasmablast (CD19+ CD20- CD3- CD38+ CD27+), classical (CD19+ IgG+ CD27+) and non-classical memory B cell (CD19+ IgG+ CD27-) sub-populations by FACS during acute infection (0–5 days after fever onset), convalescent (15 days after acute sample) and post-convalescent phases (30 days after acute sample). In parallel, using a novel strategy termed "molecular sorting", we characterized the VH repertoire in samples

of total IgG, as well as the membrane-anchored IgG+ B cell repertoire by repertoire sequencing (Rep-seq).

As previously described for dengue infection, plasmablasts peaked during the acute phase and returned to normal levels by convalescence. A similar pattern, although with less intensity was observed during acute Zika virus infection. A robust non-classical memB cell response (IgG+ CD27-), particularly secondary infections, was observed during acute dengue infection, which peaked at convalescence (d15) and persisted until post-convalescence. In contrast, in Zika virus infection showed a predominant, although less intense non-classical memB cell response. Interestingly, serum levels of CXCL13, a proposed marker of GC response (Havenar-Daughton et al., 2016), also peaked at Dengue convalescence and returned to acute levels in post-convalescence, indicating that the major germinal center activity occurs during convalescence, both in primary and secondary infections. The serum CXCL13 levels in Zika showed a similar pattern as dengue, although were less intense than dengue, and no differences between dengue-exposed and dengue-naïve patients were observed. Given that non-classical memB cells display low levels of SHM (Fecteau et al., 2006), our results strongly suggest that the plasmablast burst observed during acute phase is clonally related with non-classical memB cells and support an important contribution of the GC-i response to the overall B cell response. Taking onto consideration that symptomatic Dengue occurs 5–10 days after infection, kinetics of CXCL13 and classical memB cell response suggest a delayed GC-dependent response, particularly in secondary infections. Our currently ongoing analysis of VH region sequencing data in temporal terms, clonal relationships and SHM levels in total and membrane-anchored IgG compartments are expected to shed light of B cell differentiation pathways as a result of arboviral infection, and more importantly will reveal if SHM and B cell memory in response are impaired.

REFERENCES

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., et al. (2013). The global distribution and burden of dengue. Nature 496(7446), 504-507. doi: 10.1038/nature12060.

Fecteau, J.F., Cote, G., and Neron, S. (2006). A new memory CD27-IgG+ B cell population in peripheral blood expressing VH genes with low frequency of somatic mutation. J Immunol 177(6), 3728-3736.

Godoy-Lozano, E.E., Tellez-Sosa, J., Sanchez-Gonzalez, G., Samano-Sanchez, H., Aguilar-Salgado, A., Salinas-Rodriguez, A., et al. (2016). Lower IgG somatic hypermutation rates during acute dengue virus infection is compatible with a germinal center-independent B cell response. Genome Med 8(1), 23. doi: 10.1186/s13073-016-0276-1.

Guzman, M.G., and Harris, E. (2015). Dengue. Lancet 385(9966), 453-465. doi: 10.1016/S0140-6736(14)60572-9.

Havenar-Daughton, C., Lindqvist, M., Heit, A., Wu, J.E., Reiss, S.M., Kendric, K., et al. (2016). CXCL13 is a plasma biomarker of germinal center activity. Proc Natl Acad Sci U S A 113(10), 2702-2707. doi: 10.1073/pnas.1520112113.

Katzelnick, L.C., Gresh, L., Halloran, M.E., Mercado, J.C., Kuan, G., Gordon, A., et al. (2017). Antibody-dependent enhancement of severe dengue disease in humans. Science 358(6365), 929-932. doi: 10.1126/science.aan6836.

Development and characterization of an infection model by CagA (+) Helicobacter pylori in human B lymphocytes

Rodrigo Cervantes-Díaz[1,2,3,4], Javier Torres[3], Claudia Sandoval-Montes[4], Leopoldo Santos-Argumedo[2], José Luis Maravillas-Montero[1]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico
[3]Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias (UIMEIP), Hospital de Pediatría, Dr. Silvestre Frenk Freund, CMN Siglo-XXI, Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[4]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
rodrigo.cervantes.diaz@gmail.com; uimeip@gmail.com; claudiaqfb@yahoo.com.mx; lesantos@cinvestav.mx; maravillas@cic.unam.mx

Keywords: Helicobacter pylori, Lymphoma, Non-Hodgkin, B cells, oncoprotein, CagA

Introduction: Helicobacter pylori (a Gram-negative bacterium, with spiral shape and the approximate size of 3.5 μm) has a wide variety of virulence factors that influence the magnitude of colonization and other events related to the beginning and progression of gastric disorders such as peptic ulcer and gastric cancer.

The cytotoxin-associated gene A (or cagA) is a virulence factor that has drawn attention due to its oncogenic capacity and the association of its expression by H. pylori with pathologic effects, including gastric cancer and MALT-lymphoma; a non-Hodgkin B cell related-lymphoma that is associated with the chronic inflammatory response induced by H. pylori infection. Evidence suggests that cagA has a central role on developing MALT-lymphoma due to its "promiscuous" way to interact with signaling pathways once inside epithelial cells and it has also been described that the eradication of the pathogen with an antimicrobial therapy leads to the regression of the MALT-lymphoma. The transgenic mice model with constitutive expression of cagA displays a phenotype characterized by the presence of tumors, among other characteristics. This model sets the base to imply that cagA is the molecule that mainly disrupts gastric cells' physiology and starts the development of the MALT-lymphoma.

The information related to cagA and its association with signaling pathways upon its translocation to the cell cytoplasm has been well established only in epithelial cells, where it affects cell growth, proliferation and may cause cell death. In the case of B

lymphocytes, physiology modifications by translocated CagA have not been appropriately characterized. The study of the interaction is necessary to understand how MALT lymphomas develop from an infection with a strain of H. pylori cagA (+).

Aim: To develop a model of infection of human B lymphocytes lines with H. pylori cagA (+) strains.

Results: The translocation of cagA into B lymphocytes lines (Raji and Ramos); cultured in RPMI-1640 medium with 10% of FCS, is evident after a 6 hour-infection with a 48-hour culture of H. pylori cagA (+) at MOI of 100.The infection consists on culturing B lymphocytes with H. pylori for 6 hours, after that the culture is cleared of bacteria by incubating the infected culture with RPMI medium with gentamicin (200 ng/ml) for 2 hours. We assessed the translocation of the virulence factor by western blotting B cells lysates with and anti-cagA antibody (Santa Cruz Biotechnology sc-28368).

Conclusion: CagA is efficiently translocated into B lymphocytes after infection with an H. pylori cagA (+) strain.

Delayed proliferation and increased apoptosis in B cell lines with lrba deficiency

Daniela Pérez-Pérez[1,2], Gabriela Lopez-Herrera[1], Gabriela Lupercio-Ramírez[2], Laura Berron Ruiz[1]

[1]Instituto Nacional de Pediatria, Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
dan.pez1515@gmail.com; lohegabyqbp@gmail.com; gabylup.4@gmail.com; lberronruiz@yahoo.com.mx

Keywords: Apoptosis, B cells, proliferation, CRISPR-Cas9 system, LRBA

Introduction: LRBA is a high-molecular weight intracellular protein expressed ubiquitously and overexpressed in activated lymphocytes and macrophages. At the moment, the mechanism of this protein in B cells has not been elucidated; however, in 2012 the deficiency of LRBA was associated to Common Variable Immunodeficiency (CVID), characterized by hypogammaglobulinemia, B cell differentiation defects, and autoimmunity. Although it has been demonstrated that LRBA is involved in CTLA-4 recycling, its specific role in B-cells is still unknown). However, studies show that it could be important in activation, autophagy, and apoptosis in B cells. We aimed to obtain B cell cancer cell lines deficient in LRBA and to determine if these cell lines share B cell defects similar to those observed in CVID. Additionally we aimed to determine if LRBA deficiency shows defects in B cell receptor signaling.

Objective: To determine if LRBA deficient B cell lines exhibit functional defects similar to those observed in human primary B-cells.

Material and Methods: LRBA expression was evaluated in primary murine B-cells by flow cytometry after stimulation. A CRISPR-Cas9/LRBA plasmid was transfected through lipofectamine into the B-cell line to obtain LRBA deficient cells. LRBA deficiency was monitored by protein expression through Western Blot. The expected mutation was confirmed by sequencing exon 2 of LRBA gene. Proliferation, apoptosis, autophagy and calcium flux were assessed in LRBA deficient B cell lines and compared with its wild-type counterpart.

Results: Previous to this project, we demonstrated that murine LRBA was highly expressed in mature B cells; due to this, we aimed to introduce mutations in LRBA using CRISPR technology in a mature B cell line. Currently, a LRBA-deficient B cell

line was obtained. The lack of LRBA expression on these cells was confirmed by two methods: western blot and flow cytometry. Additionally, a missense mutation in exon 2 was observed. The functional assays showed a decrease in divided cell percentage and in division index on cells that don't express LRBA compared to wild-type cells. Furthermore, LRBA deficient B cells showed an increase in Annexin-positive apoptotic cells percentage. Contrary to this, the autophagy assays indicated lower levels of LC3-II, indicating that autophagy is decreased in LRBA-deficient B cells. Finally, calcium flux was reduced after PMA-Ionomycin stimulation in LRBA-deficient B cells.

Conclusions: LRBA deficiency in B cells shows similar functional defects to those reported in primary B cells, making possible to use them as a model to study LRBA deficiency. It will be interesting to continue studying the functional defects using a different type of stimuli in the cell line we have generated in order to find the signaling pathway in which LRBA participates in B-cells.

Differences in the transcriptome and characterization of repertoire of immunoglobulin genes of IgMhi and IgMlo human memory B cells

Diana Marcela Bautista-López[1], Andrés Camilo Vásquez Flórez[1], Juan Téllez-Sosa[2], Jesus Martinez-Barnetche[2], Manuel Antonio Franco[1], Juana Angel[1]

[1]Pontifical Xavierian University, Bogotá, Colombia
[2]Instituto Nacional de Salud Pública, Cuernavaca, Mexico
bautista.diana@javeriana.edu.co; ancavaflo@gmail.com; jmtellez@insp.mx; jmbarnet@insp.mx; mafranco@javeriana.edu.co; jangel@javeriana.edu.co

Keywords: Transcriptome, B lymphocytes, IgM, IgD, Immunoglobulin genes

We have previously shown that circulating human IgM memory B cells can be separated into CD19+ IgMhiIgDlo and CD19+ IgMloIgDhi (Narvaez et al., 2012). Here, these two subsets of B cells were FACS purified from blood of six volunteers. RNA from three of them was extracted and processed for hybridization with the Affymetrix Clariom-D array to compare their transcriptomes. RNA from others was used to amplify variable gene segments of immunoglobulins with 5'RACE-PCR and then sequenced in an Illumina Sequencer (Godoy-Lozano et al., 2016). Normalization of the data of Clariom-D was performed with the Affymetrix expression console using the RMA method and analyzed with the Affymetrix Transcriptome Analysis Console. For pathway analysis, the data was re-normalized using affy R package and clariomdhumanhsentrezgcdf R package from the Molecular and Behavioral Neuroscience Institute (Brainarray) at the University of Michigan, which maps the probes on the array to unique Entrez Gene identifiers. 39,337 probesets identifiers were found after re-normalization process and only those that had an FDRq LME-ANOVA<0.001 and P < 0,01 were selected to differential expression of genes and clustering analysis. Additionally, principal component analysis (PCA), gene set enrichment analysis (GSEA), and leading Edge analysis (LEA) were performed. On the other hand, total reads from immunoglobulin genes sequenced in MiSeq, both for heavy (IGH) and kappa light chains (IGK), were analyzed in FastQC software to evaluate their quality. Then immunoglobulin gene sequences were identified in IgRec software using Unix commands. Fasta data from IgRec reports were confirmed in IMGT database. Unique and shared clonotypes within subpopulations were further analyzed.

With the microarrays analysis, the CD19+IgMhiIgDlo and CD19+IgMloIgDhi cells are clustered together and differentiated from the naive and switched memory B cells

in the PCA analysis. The transcriptomic profile of 78 selected genes differs between CD19+IgMhiIgDlo and CD19+IgMloIgDhi cells, and 159 biological processes were identified enriched in CD19+IgMhiIgDlo cells with respect to CD19+IgMloIgDhi with an FDR <0.25, ten of which correspond to B cells. Preliminary analysis of Ig gene sequences showed that about 30,000 to 200,000 total reads from CD19+IgMhiIgDlo and CD19+IgMloIgDhi populations were recognized as immunoglobulins by IgRec. The initial and final position, length, and score of VJ genetic segment from all the samples were within the expected parameters, except for one CD19+IgMloIgDhi population. The number of clusters reported in IgRec were also found in IMGT, confirming the results. The number of clusters reported in IMGT are almost exclusively productive. The length of the CDR3 is normal for both immunoglobulin chains, unique and shared clones were found between populations, and these would allow to clarify similarities and differences between both populations.

Preliminary analyses of the transcriptomic data suggest that CD19+ IgMhiIgDlo and CD19+ IgMloIgDhi are two distinct populations. More detailed examination of the immunoglobulin genes is necessary to confirm this result.

REFERENCES

Narvaez, C. F., Feng, N., Vasquez, C., Sen, A., Angel, J., Greenberg, H. B., & Franco, M. A. (2012). Human Rotavirus-Specific IgM Memory B Cells Have Differential Cloning Efficiencies and Switch Capacities and Play a Role in Antiviral Immunity In Vivo. Journal of Virology, 86(19), 10829–10840. https://doi.org/10.1128/JVI.01466-12

Godoy-Lozano, E. E., Téllez-Sosa, J., Sánchez-González, G., Sámano-Sánchez, H., Aguilar-Salgado, A.Salinas-Rodríguez, A., … Martínez-Barnetche, J. (2016). Lower IgG somatic hypermutation rates during acute dengue virus infection is compatible with a germinal center-independent B cell response. Genome Medicine, 8(1), 1–19. https://doi.org/10.1186/s13073-016-0276-1

BAFF, BAFF-R, TACI and BCMA glandular expression analysis and its association with ectopic germinal centers formation in minor salivary glands from primary Sjögren's syndrome patients

Francisco Josué Carrillo-Ballesteros[1], Claudia Azucena Palafox-Sánchez[1], Ramón Antonio Franco-Topete[2], José Francisco Muñoz-Valle[1], Gloria Martínez-Bonilla[2], Claudia Elizabeth Gómez-López[2], Gerardo Orozco-Barocio[3], Miguel Marin-Rosales[1], Erika Fabiola López-Villalobos[1], Ernesto Alvarado-Quintero[1], Edith Oregon-Romero[1]

[1]University of Guadalajara, Guadalajara, Mexico
[2]Hospital Civil de Guadalajara, Guadalajara, Mexico
[3]Hospital de Especialidades CMN Occidente, Guadalajara, Mexico
fjosue_2111@hotmail.com; kklaumx@yahoo.com; francotra@yahoo.com; biologiamolecular@hotmail.com; glomarbon@hotmail.com; draklaudiagomez@gmail.com; gerardo.orozco@cucs.udg.mx; marin_cucs@hotmail.com; erika.f_lopez@hotmail.com; ernestoaq1@gmail.com; oregon_edith@hotmail.com

Keywords: BAFF, Sjögren's syndrome, Minor salivary gland, BCMA, TACI, BAFF-R, ectopic germinal center

Introduction: B cell activating factor (BAFF) is an essential cytokine for B cell survival, development, maturation and differentiation due to the interaction with its receptors: BAFF-R, TACI and BCMA. These processes occur mainly in germinal centers (GCs) within secondary lymphoid tissues, such as tonsils. Moreover, it has been related the presence of high BAFF serum levels with autoimmune diseases, such as, primary Sjögren's syndrome (pSS), which is characterized by a mononuclear cells focal infiltrate and a progressive damage in exocrine glands, mostly salivary and lacrimal glands. BAFF overexpression in exocrine glands from pSS patients contributes to B cell chemotaxis, survival and affinity maturation in ectopic GCs, which have been reported in about 20–30% of pSS patients, playing a role in glandular injury, autoantibodies production and disease severity.

Aim: To evaluate BAFF, BAFF-R, TACI, and BCMA expression in minor salivary glands (MSGs) from pSS patients and their association with ectopic GCs formation and clinical severity.

Methodology: MSGs biopsy and serum were obtained from 26 patients classified with pSS and 3 subjects with oral and ocular dryness who did not fulfilled all criteria for pSS. Also 9 subjects submitted to a tonsillectomy were included. Tissues were paraffin-embedded, and MSGs were classified by histopathological diagnosis as non-specific chronic sialadenitis (NSCS), focal lymphocytic sialadenitis (FLS), and another group with presence of ectopic GCs, characterized by their classical morphology and CD21 expression for the identification of follicular dendritic cells networks. Then BAFF, BAFF-R, TACI and BCMA staining was assessed by immunohistochemistry, and expression of CD3 and CD20 were used to identify T and B cells aggregates, respectively. Serum levels of BAFF, anti-Ro/SSA, anti-La/SSB, antinuclear antibodies (ANAs), and immunoglobulin G (IgG) were measured by ELISA, and rheumatoid factors (RF) were quantified by turbidimetry in pSS patients. Disease activity and severity were evaluated with SSDDI, SSDAI and ESSDAI indexes.

Results: In MSG, lymphocytic infiltrate evaluated by focus score was significantly higher in biopsies with presence of ectopic GCs than FLS, NSCS with pSS, and subjects with oral and ocular dryness (3.97 vs. 2.72, 0.63, and 0.63, respectively, p=0.001). BAFF glandular expression was increased in patients with FLS and ectopic GCs formation. Moreover, BAFF-R also showed an increased expression pattern in MSG with ectopic GCs in a similar pattern like observed in mantle zone from tonsillar follicles. TACI and BCMA glandular expressions were similar between pSS patients with FLS and those with ectopic GCs formation. Respect to BAFF, soluble levels in pSS patients were higher than healthy control subjects (1093 vs 830.7 pg/mL, p=0.032). Moreover, the highest levels of sBAFF were found in the SLF group (1252 pg/mL). Correlation analysis with all pSS data, displayed associations between sBAFF and disease evolution (r=0.4373, p=0.0289), sBAFF and anti-La/SSB autoantibodies levels (r=0.4486, p=0.0146), disease evolution and anti-Ro/SSA autoantibodies (r=0.4093, p=0.0422) and disease evolution and SSDAI score (r=0.4910, p=0.0127).

Conclusions: Our findings suggest that presence of BAFF and its receptors in salivary glands from pSS patients could contribute to the disease pathogenesis through ectopic GCs formation and maintenance, which may affect clinical severity.

Immune response mediated by B1 and B2 cells in Balb/c mice immunized with proteins from Nocardia brasiliensis

Mayra Alejandra Rodríguez-Serrato, Martha M. Solís-Treviño, Mario Cesar Salinas-Carmona, Alberto Yairh Limón-Flores

Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico
mayra_92_c@hotmail.com; marthasolis90@gmail.com; mario.salinas@uanl.mx; aylf10@hotmail.com

Keywords: Antibodies, Germinal Center, Nocardia, B1 cell, lymph node., B2 cell

Nocardia brasiliensis is an intracellular organism that produces micetoma and the immunopathogenesis behind this disease is not well understood. Researchers have shown that IgM antibodies and/or a Th1 response are essential for protection against Nocardia brasiliensis. We immunized BALB/c mice with soluble antigen p24 of Nocardia brasiliensis without adjuvant and evaluated the formation of germinal centers and production of IgM and IgG. We found that soluble antigens promoted a strong B2 cell response and B1 cell recruitment in regional lymph nodes. Recruitment started at day 9 and persisted until day 28 post immunization, and IgM antibodies were detected throughout this period, although we could not identify the cell population sustaining this response. This study demonstrates the differences in B1 and B2 cell response caused by various Nocardia brasiliensis antigens. Also, the B1 predominance and sustained IgM, IgG2a and IgG2b levels correlate with the protective role previously described.

LRBA characterization in B lymphocytes

Nidia Carolina Moreno Corona[1,2], Leopoldo Santos-Argumedo[2], Gabriela López Herrera[1]

[1]Instituto Nacional de Pediatria, Mexico City, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
caro-nidia@hotmail.com; lesantos@cinvestav.mx; lohegabyqbp@gmail.com

Keywords: Autoimmunity, B cells, AKAP, PKA, LRBA

Introduction: LPS-Responsive Beige-Like Anchor (LRBA) protein was initially identified as a LPS-up-regulated gene in B cells (Feutcher, 1997), is a novel protein with an important function in the immune system (Wang, 2014). LRBA weights 320kDA, and has 2863 amino acids, its sequence contains multiple domains which suggest this protein can act as a scaffolding protein. LRBA contains a Dinein Heavy Chain-like domain, a Concanavalin A (ConA)-like lectin binding domain, two RII binding motifs, WD-like (WDL), and Beige and Chediak-Higashi (BEACH) domains and five WD40 repeats, (Wang,2014). Most BEACH proteins are large and share a domain architecture in which the highly conserved BEACH domain is followed by a WD repeat at the C-terminus, whereas the N-terminal sequence regions are more divergent. Several BEACH proteins are implicated in human diseases (Cullinane, 2013).

As mentioned before LRBA has two binding sites to the regulatory subunits of PKA. The cAMP-dependent protein kinase A is a tetramer composed of two regulatory subunits (R unit) and two catalytic subunits (C unit). A kinase anchor proteins (AKAPs) bind protein kinase A (PKA) by inserting the hydrophobic side of the helix into the hydrophobic pocket formed by the two regulatory subunits of a PKA (Tasken, 2002). This activation leads to translocation of PKA to a distinct subcellular location where it is activated by cAMP binding. We believe LRBA can function as an AKAP.

Objective: To look for the function of LRBA as an AKAP in B lymphocytes.

Material and Methods: We worked with different B cell lymphomas such as RAMOS, DAUDI and RAJI cell lines as well as primary B cells obtained from peripheral blood (sorted with CD19 magnetic beads). We used confocal microscopy to observe colocalization of the different regulatory subunits of PKA (RIIβ, RIIα, and RIα) and LRBA. We immunoprecipitated LRBA and PKA subunits to look for interaction in these cells.

Results: First, we looked for the expression of PKA subunits and LRBA in different B cell lines and primary B cells, analyzed cells expressed the subunits (RIIβ, RIIα, and RIα). We also observed the co-localization of RIIB and LRBA in Ramos Cell line, as well as co-localization with RIIα with LRBA in primary B cells.

We co-immunoprecipitated the protein using the Ramos cell line and observed that LRBA interacts with RIIβ in these cell lines. However, when we IP LRBA in primary B cell lines, stimulated with CD40L and IL-4, we found that LRBA interacts with RIIα in these cells, suggesting that LRBA is capable of connecting with different regulatory subunits.

Conclusion: We observed that LRBA can function as a PKA anchoring protein. It can bind to RII regulatory subunits, and that interaction depends on the stimulus used.

REFERENCES

1. Feuchter, A. E., Freeman, J. D. & Mager, D. L. Strategy for detecting cellular transcripts promoted by human endogenous long terminal repeats: Identification of a novel gene (CDC4L) with homology to yeast CDC4. *Genomics* **13,** 1237–1246 (1992).
2. Wang, J. & Lockey, R. F. Lipopolysaccharide-Responsive Beige-like Anchor (LRBA), a Novel Regulator of Human Immune Disorders. *Austin J. Clin. Immunol.* **1,** 1–9 (2014).
3. Cullinane, A. R., Schäffer, A. A. & Huizing, M. The BEACH Is Hot: A LYST of Emerging Roles for BEACH-Domain Containing Proteins in Human Disease. *Traffic* **14,** 749–766 (2013).
4. Torgersen, K. M., Vang, T., Abrahamsen, H., Yaqub, S. & Tasken, K. Molecular mechanisms for protein kinase A-mediated modulation of immune function. *Cell Signal* **14,** 1–9 (2002).

A potential role for mitochondrial Ca2+ overload in B cell fate determination

Alejandro Torres-Quintanilla, Elena C. Castillo, Gerardo García-Rivas

Monterrey Institute of Technology and Higher Education, Monterrey, Mexico
atorresq@itesm.mx; ecgonzalez@itesm.mx; gdejesus@itesm.mx

Keywords: differentiation, B lymphocyte, ROS, CD69, Mitochondrial calcium overload

Mitochondria have been described as key players of immunometabolism, regulating cell function through metabolic control. Through Ca2+ uptake, mitochondria regulate different cell functions like Ca2+ handling, metabolic output and cell death. Indeed, recent evidence suggests that mitochondrial signals determine the fate of B cell differentiation. However, the specific mechanisms for mitochondrial contribution to B cell activation are not completely understood. Here, we propose that continuous Ca2+ uptake after B cell activation leads to mitochondrial Ca2+ overload and cell death, and cells capable of surviving this Ca2+ surge differentiate into long-lasting memory cells. To explore this, an in vitro model of mitochondrial Ca2+ overload by thapsigargin treatment was generated. Secondly, the expression of activation marker CD69 was determined on cells subjected to mitochondrial Ca2+ overload. Mitochondrial Ca2+ uptake or mROS production was inhibited by Ru360 or MitoTEMPO treatment. Finally, mitochondrial Ca2+ overload impact on B cell activation through BCR was explored. Evaluation of the effect of mitochondrial Ca2+ overload on the expression of differentiation markers is pending. Our results show that thapsigargin treatment abolishes mitochondrial membrane potential ($\Delta\Psi$)m and increases mitochondrial Ca2+ content. Inhibition of mitochondrial Ca2+ uptake or mROS production, after thapsigargin-induced mitochondrial Ca2+ overload or BCR-dependent activation, reduces the expression of activation marker CD69. Our results suggest that B cell activation signals are influenced by mitochondrial Ca2+ overload, opening the possibility for Ca2+ overload to influence fate determination.

Characterization of cytokine-producing B cell subsets in human tonsils, insights into their role in tonsillar hypertrophy and recurrent tonsilitis

Lindybeth Sarmiento Varon[1], Javier De Rosa[1], Mercedes Montani[1], Bibiana Paoli[2], Maria Elena Arabolaza[2], Pablo Fernandez[1], Karina Alejandra Pasquevich[3], Eloisa Irene Arana[1]

[1]Instituto de Inmunología, Genética y Metabolismo (INIGEM-CONICET), Hospital de Clínicas "José de San Martín", Universidad de Buenos Aires, Buenos Aires, Argentina
[2]Hospital de Clínicas "José de San Martín, Buenos Aires, Argentina
[3]National University of General San Martín, San Martín, Buenos Aires, Argentina
lindysarmiento6@gmail.com; derosajavier@gmail.com; ma.montani@gmail.com; bibianapaoli@uolsinectis.com.ar; marabolaza50@yahoo.com.ar; pfernandez1120@gmail.com; kpasquevich@gmail.com; eloarana@yahoo.com

Keywords: mucosal immunity, regulatory B cells, oral tolerance, Disregulated germinal center, Tonsillar disease

Tonsils are secondary lymphoid organs which must grant immunologic protection against pathogens and tolerance to harmless antigens (Ags) in air and food. They are mostly B-cell (Bc) maturation and differentiation sites. Bc have traditionally been associated with antibody production and Ag presentation. Lately, the Bc field has been transfigured by the attribution of new functions. For instance, Bc secrete multiple cytokines under different stimuli. It is likely that alteration of the immune equilibrium activation/regulation may trigger recurrent tonsillitis (RT) and/or hypertrophy (HT) leading in turn to tonsillectomy. Hence, the aim of our work was to characterize those unconventional immune functions of tonsillar Bc, i.e different Bc subsets with regulatory and pro-inflammatory profiles, and their association with the disease that led to surgery. Methodology used involved FACS and real time PCR. Initially,we determined tonsillar Bc expression of IL17, IL6, IL8, IFNg, IL5, TNFa, IL10 and TGFb for different patients upon three sets of stimuli (IL2+IL4/ IL2+IL4+CpG+CD40L/ aIgM+CD40L). Moreover, we established the kinetics of appearance of IL10 (regulatory cytokine) expressing Bc (Breg) and compared it with that of pro-inflammatory cytokine-expressing Bc (IL6 and IL8) for 5 patients upon 0, 16, 32 and 72 hs stimulation, via TLR9 and CD40. The proportion of Breg increased between 16 and 72 hs in all cases. Notably, the percentage of Breg detected at the different time points depended on the cause of surgery. The hypertrophied (HT) tonsils´ samples showed a significantly ($p < 0.05$) lower percentage of Bregs (4.4±0.4) compared to those from recurrent tonsillitis (RT, 9.9±1.5) as well as a significantly ($p < 0.05$) higher proportion of the germinal center (GC) population (23.8±4.3) than those from children with RT (7.9±1.8). A comparative

increment of the GC percentage was not accompanied by a proportional growth of the memory B cell population in those HT samples. In contrast, these samples displayed a significantly ($p < 0.05$) lower percentage of the eBm5 subset (7.8±0.5) than RT samples (15.5±2.1), indicative of a putative blockade between the GC and memory B cell stages on HT patients. Collectively, our results demonstrate that a defective tonsillar Breg compartment indicates an increase in the proportion of GC in vivo and therefore unrestrained T follicular helper (Tfh) function, suggesting that Tfh are a target population of Breg function. Interestingly, Breg modulated cytokine intracellular expression and proliferation of stimulated CD4+ cells, in co-cultures performed with those autologous sorted subsets at 3 different ratios. Finally, we found Bregs at every stage of B cell diferentiation represented in tonsils, that is, Bregs resulted diffusely scattered throughout the B cell lineage. Our findings provide greater insight into the role of Bregs in GC reactions and characterize for the first time their involvement in the pathogenesis of tonsillar dysfunction. Moreover, we noticed a relevant proportion of IL5 and IL17-expressing B cells in tonsils which is, to our knowledge, the first time that is reported in literature. We consider this work holds relevant implications for basic immunology as well as for translational medicine. In terms of basic immunology, human B cells with regulatory function remain poorly understood. Our results shed some light on the controversy of their phenotype and support the notion that they are indeed a separate lineage, in addition to confirm their novel immune function at the GC reaction. As for translational medicine, there is a clear need for more extensive immunological studies focusing on palatine tonsils. It is unfortunate that their immunologic role is not yet completely understood, in particular having in mind not only that infectious and inflammatory diseases involving the pharynx, tonsils, and adenoids account for a significant proportion of childhood illnesses and pediatric health care expenditures, but also that the oral/nasal route for vaccine administration has proved highly relevant to stimulate both mucosal and systemic immunity.

New insights of TSPAN33 function in B-cells

Ernesto Acevedo-Ochoa[1,2], Zayda Lizbeth Piedra-Quintero[3], David Eduardo Meza Sánchez[2], Rodrigo Cervantes-Díaz[2,3], Leopoldo Santos-Argumedo[3], Constantino III Roberto López-Macías[1], José Luis Maravillas-Montero[2]

[1]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[3]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
erez128@gmail.com; zpiedra@cinvestav.mx; tunatitlalli@gmail.com; mowrcd19@gmail.com; lesantos@cinvestav.mx; constantino@sminmunologia.com; maravillas@cic.unam.mx

Keywords: Tetraspanins, Notch, B-cells, Tetraspanin-enriched microdomains, TSPAN33

Background: Tetraspanins form highly-conserved superfamily of proteins involved in the regulation of diverse functions in different cell contexts, all related to their capacity to coordinate the spatio-temporal localization of other molecular partners in cell membranes(1). Originally, tetraspanins were described as surface antigens with some members having a wide expression pattern in tissues, while others like CD37 are only expressed by leukocytes. Moreover, these patterns have shown to dynamically change depending on the maturation stage, microenvironment or stimulus given. TSPAN33 was firstly identified in mouse erythroblasts, it shares >90% homology with its human orthologue and has a restricted expression pattern that includes erythroid cells and activated leukocytes (2-7). The expression at the mRNA level can be induced with different stimulus, consistently, TSPAN33 is constitutively expressed in B-cell associated diseases like lupus(2,3,7). Along with other tetraspanins, it forms a subgroup called TspanC8 characterized for having a highly-conserved LEL which associates with ADAM10 and regulate its trafficking. Previous studies have linked TSPAN33 with Notch signaling, tissue development and immune response against pathogens in invertebrates and mollusks, thus, supporting the hypothesis of its requirement in higher vertebrates (3,4,5). Although it is one of the two TspanC8 having a knock-out (KO) mouse model, the different expression pattern in humans and the lack of reliable antibodies have restricted the study of its functions to the use of tagged models. Here, we report the generation of a TSPAN33 KO model human B-cells (Raji) and, by using a set of antibodies along with a transgenic model we further investigated several properties like subcellular localization, expression kinetics and its association with other tetraspanins, ADAM10 and Notch.

Materials and Methods: To detect TSPAN33, we validated the use of different antibodies for applications like flow cytometry, stimulation, immunofluorescence and blotting. Shortly after, KO Raji cells were generated using CRISPR-Cas9 together with three non-overlapping sgRNA's that were cloned into vectors and delivered by microporation into Raji cells. Subsequently, cells were screened for genomic deletions with PCR and validated by western-blot to obtain a fully-edited clone.

Native localization of TSPAN33 was analyzed in Raji WT cells using markers for different intracellular compartments and organelles. Formation of immunological synapses with superantigens were also performed to observe fast membrane recruiting and polarization during antigenic-stimulus. Finally, association with other tetraspanins and Notch receptors was analyzed through immunoprecipitation, flow cytometry and confocal microscopy.

Results: In Raji cells under normal culture conditions, TSPAN33 is mainly kept in intracellular compartments and only after stimulus like IL4/αCD40, it can be detected in the surface of these cells, consistently, the same pattern was observed in PBMC's. Moreover, TSPAN33 is confined to vesicles associated to the Golgi apparatus and it is recruited to the surface into structures like microvilli and exosomes, accordingly, we observed depletion of this vesicles during immunological synapse formation. Also, although the surface levels of TSPAN33 measured in stimulated WT cells were similar to those detected in the GFP-tagged clone used, the presence of the tag modified its spatio-temporal localization independently of stimulus.

The normal etraspanin repertoire was also analyzed in the GFP-tagged model, strikingly, these cells showed changes in the surface levels of various tetraspanins including CD9, a marker that has been always reported as negative for these cells. Consistently, stimulation of WT cells showed that, indeed they change their normal tetraspanin repertoire upon stimulation and the absence of TSPAN33 can alter this behavior. These observations again show that cells readily change their tetraspanin repertoire depending the cellular context and this helps to initiate different changes in the biochemical and mechanical properties of their membranes in response to stimulus.

Previous studies have shown a clear link between Notch and TSPAN33 expression, but none have confirmed a clear regulation mechanism. Interestingly, forced expression of TSPAN33 in B-cells inhibited cellular proliferation and the production of IL10, moreover, despite the augmented presence of ADAM10 we could detect an overall surface accumulation of Notch receptors. Consistently, absence of TSPAN33 showed to reduce maturation and trafficking of ADAM10 to the surface before and after activation, but alterations in Notch levels could be found only when cells were stimulated.

This observation led us to analyze the interaction of the receptors with the tetraspanin-web using immunoprecipitation showing that Notch receptors can readily interact with tetraspanins. Moreover, antibody cross-linking of Notch induces polarization of tetraspanins furtherly showing their spatial association with the receptors. Finally, by using various reporters we are expecting to confirm that the sole presence of TSPAN33 negatively regulates Notch signaling but this can change after stimulation, accordingly, we expect that the absence of TSPAN33 will inhibit the activity of Notch during activation.

Conclusion: For the first time we have characterized the native behavior of TSPAN33 in B-cells by generating a KO model and using different antibodies, moreover, we also showed that TSPAN33 expression can regulate the expression and trafficking of other tetraspanins. Furthermore, we demonstrate that Notch receptors can associate with TEM's and the possible requirement of a stimuli for TSPAN33 to be a promoter of its activity. Altogether this data contributes to propose a novel model for the regulation of ADAM10 through TSPAN33 including coordination of its spatio-temporal localization and changes in its conformation or clustering during activation.

REFERENCES

1. Hemler, M. E. (2005). Tetraspanin functions and associated microdomains. Nature Reviews. Molecular Cell Biology, 6(10), 801–11. http://doi.org/10.1038/nrm1736
2. Heikens, M. J., Cao, T. M., Morita, C., DeHart, S. L., & Tsai, S. (2007). Penumbra encodes a novel tetraspanin that is highly expressed in erythroid progenitors and promotes effective erythropoiesis. Blood, 109(8), 3244–3252. http://doi.org/10.1182/blood-2006-09-046672
3. Ruiz-García A., López-López S., García-Ramírez M. J., Baladrón V., Ruiz-Hidalgo J. J., López-Sanz L., Ballesteros Á., Laborda J., Monsalve E. M., Díaz-Guerra J. M. (2016). The Tetraspanin TSPAN33 Controls TLR-Triggered Activation through Modulation of NOTCH Signaling. The Journal of Immunology.197, 3371-3381. http://doi.org/10.4049/jimmunol.1600421
4. Priyathilaka, T. T., Bathige, S. D. N. K., Herath H. M. L. P. B., Lee, S., Lee, J. (2017). Molecular identification of disk abalone (Haliotis discus discus) tetraspanin 33 and CD63: Insights into potent players in the disk abalone host defense system. Fish Shellfish Immunology. 69, 173-184. http://doi.org/10.1016/j.fsi.2017.08.020.
5. Dornier, E., Coumailleau, F., Ottavi, J., Moretti, J., Boucheix, C., Mauduit, P., Schweisguth, F., Rubinstein, E. 2012. TspanC8 tetraspanins regulate ADAM10/Kuzbanian trafficking and promote Notch activation in flies and mammals. J Cell Biol. 199(3):481-96. http://doi.org/10.1083/jcb.201201133
6. Jouannet, S., Saint-Pol, J., Fernandez L., Nguyen, V., Charrin, S., Boucheix, C., Brou, C., Milhiet, P. E., Rubinstein, E. (2016). TspanC8 tetraspanins differentially regulate the cleavage of ADAM10 substrates, Notch Activation and ADAM10 membrane compartmentalization. Cellular and Molecular Life Sciences. 199(3) 481-496. http://doi.org/10.1083/jcb.201201133
7. Luu, V. P., Hevezi, P., Vences-Catalan, F., Maravillas-Montero, J. L., White, C. A., Casali, P., … Zlotnik, A. (2013). TSPAN33 is a novel marker of activated and malignant B cells. Clinical Immunology (Orlando, Fla.), 149(3), 388–99. http://doi.org/10.1016/j.clim.2013.08.005

The absence of CD38 and CD19 proteins impairs the fertility and survival in a model murine

Carlos Arturo Gallardo Hernandez[1], Hector Romero[2], Juan Carlos Rodriguez Alba[1]

[1]Universidad Veracruzana, Xalapa, Mexico
[2]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico
carlos_26_arturo@hotmail.com; hromero@cinvestav.mx; jcra19@yahoo.com

Keywords: Autoimmunity, B cells, CD19, CD38, Immunodeficiency.

The autoimmune and immunodeficiencies disorders currently represent a serious health problem as there is no specific treatment that can control them, paradoxically, given their immunocompromised state, patients with immunodeficiency are also at significantly increased risk of autoimmune disorders. In this sense, CD38 and CD19 proteins play an important role in the regulation of immune system. CD38 is a transmembrane protein widely expressed in cells of immune system, this protein is an ectoenzyme and receptor possessing key physiological roles. As ectoenzyme, CD38 generates two potent Ca2+ releasing signal metabolites, cyclic ADP-ribose (cADPR) and NAADP+, as receptor has been related in processes of proliferation, activation, differentiation and apoptosis in many types of immune cells. It has been suggested that the CD38 absence exacerbates the development of an autoimmune phenotype in a model murine, then its presence could be needed in order to regulate the autoimmunity. By other hand, CD19 is an essential molecule in the BCR signaling. Several reports show that the lack of CD19 causes a deficiency in proliferation, clonal expansion and differentiation of B lymphocytes, developing an immunodeficient phenotype in mice lacking the protein. Correspondingly, some reports have suggested an association between the CD19-CD38 signaling pathways, in previous reports of our group we analyzed the CD38 expression on B cells from spleen and bone marrow in the CD19-deficient mice in the B6 background, our results showed a decrease in the CD38 expression in B lymphocytes from peripheral lymphoid organs (figure 1). Consequently, we propose that the CD38 decreased in B cells from CD19 deficient mice is due to a disruption in the interaction CD38-CD19 and/or their signaling molecules. Furthermore, it is needed to elucidate the mechanism of this interaction. In order to study the development of autoimmune and immunodeficiencies disorders in a similar way to how they occur in the human being, we have generated a new murine model deficient in the expression simultaneous of CD38 and CD19. The double knockout mice were generated based on the reported cross-over protocols of Jackson Laboratory (figure 2). We found that the double knockout mice have low reproductive capacity, previous reports show that the

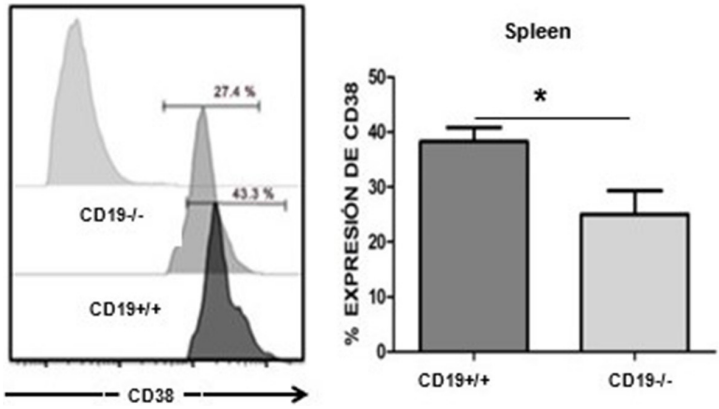


FIGURE 1: Expression of CD38 in splenic B cells from CD19$^{+/+}$,. and CD19$^{-/-}$ mice. We observed that CD38 expression it was higher in the CD19$^{+/+}$ mice compared with CD19$^{-/-}$ mice. Isotype control staining for the pairs used in each combinaion. Splenic cells from CD19$^{+/+}$ and CD19$^{-/-}$ were 4 months old age. p= 0.038

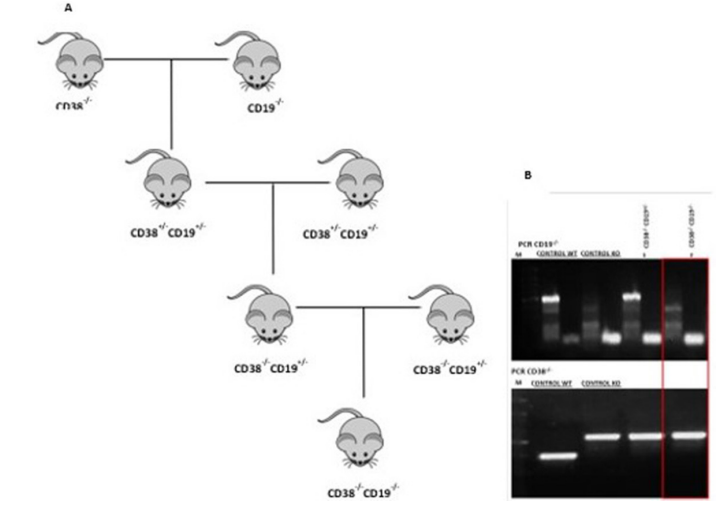


FIGURE 2: Generation of double knockout mice. A) The double knockout mice was generated crossover the CD38 knockout mice with the CD19 knockout mice, from this cross will be heterozygous for both knockout alleles. The heterozygous were cross together from this cross, we identify the genotype CD38$^{-/-}$ CD19$^{+/-}$ and were cross together for generate the CD38$^{-/-}$ CD19$^{+/-}$ mice. B) The genotype was performed with PCR for CD38 and CD19 knockout mice, in the red square show the genotype of the double knockout mice.

absence of CD38 increases the abortion rate and that a soluble form of CD38 (sCD38) released from seminal vesicles to the seminal plasma acts as an immunoregulatory factor to protect fetuses from maternal immune responses moreover sCD38 present in seminal plasma plays a critical role in the capacitation of sperm. Additionally, has been reported that CD38 knockout mice has low levels of oxytocin resulting in an impairment of maternal behavior and male social recognition in mice, our data shown fertility problems in these specific mice, furthermore we found that the absence of CD38 and CD19 shortens survival, dying around 3 months of age. In the same way, the body weight of the double knockout mice was evaluated and we found that these mice have a low body weight compared to the Wilde Type mouse. As perspectives, it is necessary to evaluate characteristic signs and symptoms of immunodeficiency and autoimmunity to observe the complete phenotype of this model and confirm its usefulness in the study of immunological disorders. Furthermore, this model can be useful to observe the interaction in the signaling pathways between CD38 and CD19.

Tetraspanin 33 (TSPAN33) regulates adhesion and chemotaxis of human B lymphocytes by affecting the composition and tension of the plasma membrane

Itze Cecilia Navarro-Hernandez[1,2], Orestes López-Ortega[3], Ernesto Acevedo-Ochoa[1], César Perez-Martinez[3], David Eduardo Meza-Sánchez[1], Arturo Galván-Hernández[4], Iván Ortega-Blake[4], Armando Antillón[4], Bibiana Chávez-Munguía[2], Rogelio Fragoso-Soriano[2], Leopoldo Santos-Argumedo[3], José Manuel Hernández-Hernández[2], José Luis Maravillas-Montero[1]

[1]Coordinación de la Investigación Científica (UNAM), Ciudad de Mexico, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
[3]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico
[4]Instituto de Ciencias Físicas, Universidad Nacional Autónoma de México, Cuernavaca, Mexico
itze.navarro9@gmail.com; olopezortega@outlook.com; erez128@gmail.com; ca_perez@hotmail.com; tunatitlalli@gmail.com; crazyarthur@gmail.com; ivan@fis.unam.mx; armando@fis.unam.mx; bchavez@cinvestav.mx; rogelio@fis.cinvestav.mx; lesantos@cinvestav.mx; manolo@cell.cinvestav.mx; maravillas@cic.unam.mx

Keywords: Cytoskeleton, tetraspanin, plasma membrane, membrane tension, B-lymphocyte

Introduction: B-cells have arisen as specialized leukocytes capable of developing multiple functions apart from antibody production including: chemotaxis/migration, antigen presentation, cytokine production and endocytosis/phagocytosis. Most of the times, these processes are initiated by external signals that activate the cell and trigger changes in the compartmentalization, curvature and elasticity of the plasma membrane. These changes are regulated by the formation of different subdomains in the lipid bilayer which restrict the lateral mobility of lipids and proteins like adhesion molecules and receptors. Furthermore, the association of these domains with the actin cytoskeleton through different molecules allows the functional specialization of specific regions within the membrane. Tetraspanins are a superfamily of cholesterol-binding proteins capable of modifying the membrane curvature and laterally associate with other molecules regulating its localization and trafficking.

Aim: Demonstrate that TSPAN33 is an active intermediate during different adhesion and actin remodeling events in B-cells including: membrane protrusions formation, substrate adhesion/spreading, chemotaxis, invasion, and phagocytosis.

Results: By using a transgenic model of TSPAN33 in Raji cells we identified a diminished phagocytic capacity towards E. coli and an altered adhesion ability to different substrates accompanied by a reduced expression of β1 and β2 integrins. Strikingly, these cells presented a migrating / invading phenotype which included an augmented chemotaxis and invasion capacity jointly with changes in the membrane protrusions formation and exocytosis of microvesicles. Also, we found a higher roughness and membrane tension.

Conclusion: We propose that these changes are partly generated through alterations in the membrane tension and topology, putting a novel mechanical-signal mechanism for the regulation of changes in the cytoskeleton organization driven by tetraspanins in cell membranes.

3D-migration of B lymphocytes is reduced in the absence of Myosin 1g

Orestes López-Ortega[1], Alan Mauricio González-Suarez[2], Jose Luis Garcia-Cordero[2], Leopoldo Santos-Argumedo[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
[2]Unidad Monterrey, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Monterrey, Mexico
olopezortega@outlook.com; alan.bim@outlook.com; josegc@gmail.com; lesantos@cinvestav.mx

Keywords: Actin Cytoskeleton, Myosin, B lymphocyte, Microchannel, 3D-migration

Introduction: Migratory cells, including B lymphocytes, produce a single leading pseudopodium in the direction of a chemoattractant. Migration has mostly been explored using 2D approaches; however, in recent years, the use of state-of-the-art procedures (microchannel fluidic devices) has allowed a more detailed analysis of this phenomenon. Cell migration depends on cell activation and the formation of membrane-protrusions, which are regulated by the actin-cytoskeleton and class II myosins. However, the role of class I myosins is poorly described.

Myo1g is a class I myosin exclusively found in hematopoietic cells. Its expression varies between the different hematopoietic cell lineages, with the highest expression in B cells and activated T cells. Myo1g is a molecular motor that associates, in an ATP-dependent manner, to the actin cytoskeleton. It localizes to the plasma membrane and is particularly enriched at microvilli of T and B lymphocytes. Myo1g is present in lipid rafts of B lymphocytes and neutrophils and binds to phosphatidylinositol-3,4-bisphosphate and phosphatidylinositol-3,4,5-triphosphate in the plasma membrane. Myo1g is also found associated with several vesicles and controls their traffic and release. Myo1g is also involved in the maintenance and regulation of plasma membrane tension. However, little is known about its role in cell migration.

Objective: To elucidate the role of Myo1g in the adhesion and migration of B lymphocytes.

Results: We used bio-microfluidic devices to determine whether Myo1g-deficiency alters the migration of B-lymphocytes. We noted that the lack of Myo1g reduced adhesion to the fibronectin coated-microchannel chamber, under constant flow and,

under a chemokine gradient. Disturbed adhesive interactions also led to higher rolling velocities.

We show that Myo1g had a crucial role in the production of membrane-projections in response to chemoattractant stimuli, allowing the recycling of adhesion molecules and lipid rafts to a leading pole and stabilizing the membrane tension of this site. We detected Myo1g in membrane projections of B cells by western blot and confocal microscopy. Moreover, Myo1g-deficient B lymphocytes had shorter membrane-projections compared with WT B lymphocytes.

Conclusion: Our results indicate that Myo1g is a vital motor protein critical for the formation and establishment of membrane-protrusions during 3D migration.

Pd-l1+ regulatory B cells are significant decreased in rheumatoid arthritis patiens and increase after good treatment response

Estefanía Raquel Zacca[1], Luisina Inés Onofrio[1], Cristina Acosta[1], Paola Virginia Ferrero[1], Sergio Alonso[1], Maria Cecilia Ramello[2], Eduardo Mussano[1], Laura Onetti[1], Ignacio Isaac Cadile[1], María Carolina Taboada Bonfanti[1], Carolina Montes[2], Eva V Acosta Rodriguez[2], Adriana Gruppi[2]

[1]Hospital Nacional de Clínicas, Córdoba, Argentina
[2]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI CONICET), Cordoba, Argentina
ezacca@fcq.unc.edu.ar; lonofrio@fcq.unc.edu.ar; cristinacosta68@hotmail.com; paola.paolaferrero@gmail.com; ferreropaola@hotmail.com; ceciliaramello@gmail.com; edemussano@yahoo.com.ar; lauraonetti@gmail.com; ignaciocadile@hotmail.com; reumahnc@fcm.unc.edu.ar; cmontes@fcq.unc.edu.ar; eacosta@fcq.unc.edu.ar; agruppi@fcq.unc.edu.ar

Keywords: Autoimmunity, Rheumatic Diseases, Rheumatoid arthritis, PD-L1, Bregs

Background: It has been demonstrated that B cells play an important role in the development and maintenance of rheumatoid arthritis (RA). Although IL-10–producing B cells represent a major subset of regulatory B cells (Bregs) studied that suppress autoimmune and inflammatory responses, recent reports have also shown that immune suppression independent of IL-10 also occurs. For instance, it has been reported that B cells can modulate T cell immune responses through the expression of regulatory molecules such as PD-L1, however PD-L1-expressing B cells have not been analyzed in RA patients.

Objective: To analyze the frequency of PD-L1-expressing B cells in peripheral blood of RA patients compared to matched-healthy controls (HC), their function on T cell response and their changes in response to therapy.

Methods: Fresh peripheral blood B cells from 69 RA patients and 25 HC were characterized by flow cytometry and their functionality was assessed in a co-culture system with autologous T cells.

Results: The frequencies of CD19+PD-L1+ B cells, CD24hiCD38-PD-L1+ and CD24hiCD38hiPD-L1+ B cells were significantly decreased in untreated RA patients compared to HC ($p<0.01$). In a follow-up study, the frequencies of PD-L1+ B cells (CD19+PD-L1+ B cells, CD24hiCD38-PD-L1+ and CD24hiCD38hiPD-L1+ B cells) were significantly increased after treatment in good responder patients ($p<0.01$), although the frequency of total CD24hiCD38hi B cells decreased ($p<0.01$). CD19+ B cells from untreated RA patients and HC were equally able to up-regulate PD-L1 expression upon stimulation with CpG plus IL-2 and were able to suppress CD8+ T cell proliferation and cytokine production in a PD-L1-dependent manner ($p<0.05$).

Conclusions: Our results show that T cell suppressive-PD-L1+ B cells are significantly decreased in untreated RA patients but increases in response to successful treatment. PD-L1 expression on B cells from RA patients can be in vitro modulated and thereby, PD-L1+ B cells could provide new perspectives for future treatment strategies.

Myosin 1g, in collaboration with GTPases of the Rho family, participate in the recycling of CD44 present in the lipid rafts of B lymphocytes

Carlos Emilio Miguel Rodríguez, Leopoldo Santos-Argumedo, Orestes López-Ortega

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
carlos_emilio_13@hotmail.com; lesantos@cinvestav.mx; Olopezortega@outlook.com

Keywords: B cells, RhoA, CD44, GTPases, myosin 1g

Introduction: Myosins are a family of motor proteins, which, through the hydrolysis of ATP and structural changes, generate the necessary force to move along the actin filaments. Class I myosins are classified into short-tailed and long-tailed myosins. In mammals, there is eight class I myosins (six short-tailed and two long-tailed).

Myosin1g is a hematopoietic short-tailed myosin, which binds (through its tail) to the phosphoinositides PIP2 and PIP3 present in various types of vesicles. We previously analyzed the participation of Myo1g during the migration and cellular adhesion of B lymphocytes, in both phenomena the rearrangement of the actin cytoskeleton is essential, as well as the transport and recycling of adhesion molecules. During the recycling of adhesion receptors, many molecules participate in the phenomena. Proteins such as actin, tubulin, kinesins, dyneins, and GTPase, as well as lipids rafts, collaborate in recycling; however, the role of myosins is unknown.

Aim: To identify the role of Myo1g in the recycling of CD44.

Results: Myo1g deficiency affects the polarization of CD44 in B cells: We stimulated B cells 48 h with LPS + IL-4 and then incubated with the anti-CD44 monoclonal antibody (NIM-R8) to induce polarization. Confocal microscopy images demonstrated that CD44 had reduced mobility to the site of polarization in cells lacking Myo1g.

The absence of Myo1g redistributes CD44 from the cell surface to intracellular membranes: Myo1g is present in specialized lipid domains at the plasma membrane, during cell migration, CD44 is associated with microdomains. In sharp contrast, LPS +

IL4-activated B cells from Myo1g–/– had less CD44 on the plasma membrane, with accumulation inside the cells.

Myo1g participates in the recycling complex of CD44: One crucial step in the recycling process is the association of GTPases with the recycling complex. Therefore, we immunoprecipitated total lysates of B cells with anti-Myo1g antibodies. The samples were separated by SDS-PAGE, transferred to nitrocellulose, and developed with antibodies against CDC42, RhoA, Rab5, and Rab11. The results demonstrated the presence of several GTPases in the immunoprecipitates of Myo1g.

Conclusion: In this work, we identify Myo1g as an essential motor protein that directs the recycling of CD44, in cooperation with GTPases of the Rho family, in B lymphocytes. We detected the presence of Myo1g in the transport complex of CD44 with GTPases of the Rho family. Besides, we registered a delay in the activation of the GTPase RhoA due to the lack of Myo1g during the recycling of CD44. As a whole, these data suggest that Myo1g participates in the recycling of CD44 present in the lipid rafts of the plasma membrane, in collaboration with GTPases of the Rho family.

Correspondence

Dr Leopoldo Santos Argumedo, Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional. Av. IPN # 2508, Colonia Zacatenco, México 07360, D.F., México. E-mail: lesantos@cinvestav.mx

Differential expression patterns of secreted Frizzled related protein genes in different stages of Rheumatoid Arthritis

Eduardo Rodriguez-Varela[1,2], Dania Rubí Mejía-López[3], Cristian Montoya-Vazquez[3], Mariela Gómez Moreno[1,2], Leonor Enciso-Moreno[2], Julio Enrique Castañeda Delgado[1,2], Jose Alfredo Méndez[1], Martin Zapata-Zuñiga[2], José Dionisio Castillo-Ortiz[5], Cesar Ramos-Remus[5], Jose Antonio Enciso-Moreno[2], Yadira Bastian[2,4]

[1]Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico
[2]Unidad de Investigación Biomédica de Zacatecas (IMSS), Zacatecas, Mexico
[3]Autonomous University of Zacatecas, Zacatecas, Mexico
[4]Cátedras CONACYT, IMSS, ZACATECAS, Mexico
[5]Unidad de investigación en enfermedades Crónico/Degenerativas, Guadalajara, Mexico
erv_24yomi@yahoo.com.mx; erv_24yomi@yahoo.com.mx; lovelife_26daruml@outlook.es; chris_crazy@hotmail.com; mgm_09_92@yahoo.es; mgm_09_92@yahoo.es; leonorenciso1@hotmail.com; julioenrique_castaeda@yahoo.com.mx; julioenrique_castaeda@yahoo.com.mx; jamendez@ifisica.uaslp.mx; martinzz24@icloud.com; drcastilloortiz@gmail.com; r_ramos@prodigy.net.mx; enciso_2000@yahoo.com; yito21@yahoo.com; yito21@yahoo.com

Keywords: Autoantibodies, Wnt Signaling Pathway, Rheumatoid arthritis, Sfrp5, sFRP2

Rheumatoid arthritis (RA) is an inflammatory, systemic and chronic disease that affects 1.6% of the Mexican population. This disease is characterized by the symmetrical affection of the synovial membrane, cartilage and bone. Although the etiology is unknown, the main hypothesis is that RA implies a deleterious association between several processes of both genetic and environmental origin (1). Distinctive of RA is the presence of two autoantibodies, the rheumatoid factor (RF) and the anti-citrullinated peptide antibody (ACCP) as well as of some inflammatory cytokines like TNF-α, RANKL, IFN-γ IL-1, IL-6 (2, 3). Wnt signaling pathways have been involved in RA because their activation results in production of inflammatory cytokines, growth factors and proteinases (1). The Wnt signal pathway is one of the important signal transduction mechanisms related to cell differentiation in embryogenesis and hematopoiesis. This signaling pathway is tightly regulated by extracellular regulators that prevent the activation of Wnt receptors either by sequestering Wnt proteins or antagonizing their receptors. An example of both inhibition mechanisms are the Secreted frizzled related proteins (sFRP), they comprise a family of proteins that inhibit the Wnt signaling pathway by competitively interacting with Wnt proteins or FZD receptors (4). Imai K et al., compared the expression pattern of sFRPs in the synovium of patients with RA and osteoarthritis (OA), and found a predominant expression of sFRPs in OA (5).

We hypothesized that combined analysis of sFRP genes could serve as biomarkers for staging and prognosis of RA. The goal of this work is to analyze the gene expression of sFRP family members during the establishment of RA.

METHODS:

Sample Collection

4 ml of whole blood were collected into EDTA-coated tubes, 1ml of RNAlater (Invitrogen) was added and samples were immediately stored. RA patients fulfilling the 2010 American College of Rheumatology/European League Against Rheumatism. Patients were classified in two groups, early RA (less than a year with symptoms) and chronic or established RA (more than one year with symptoms). Also, First-degree relatives of RA patients with positive serum levels of ACCP (ACPA+) were included.

RT-qPCR

RNA from blood samples was isolated following a customized TRIzol-QIAamp protocol (11). cDNA was synthesized using total RNA and Superscript II reverse transcriptase (Invitrogen, USA), treated with RNaseH (Invitrogen) and stored at -20°C. Using Sso Fast-EvaGreen® (Bio Rad) in the Light Cycler 480 equipment (Roche, Switzerland), RT-qPCR was then performed for the determination of the gene expression values of sFRP-1, sFRP-2, and sFRP-5. HPRT was used as the reference gene. RT-qPCR data was analyzed by Kruskall-Wallis and Dunn´s post-test. In addition, an analysis of correlations between the analyzed genes and various parameters such as age, ACPA serum levels and Anti-CarbP antibodies levels were performed with the software SPSS version 18 (Microsoft, USA).

Results: Of the 54 RA patients (including ACPA+ subjects), 44 were female, 7 male, and the median age was 43.5 years. Of the 21 healthy controls, 14 were female, 7 male, and the median age was 43 years.

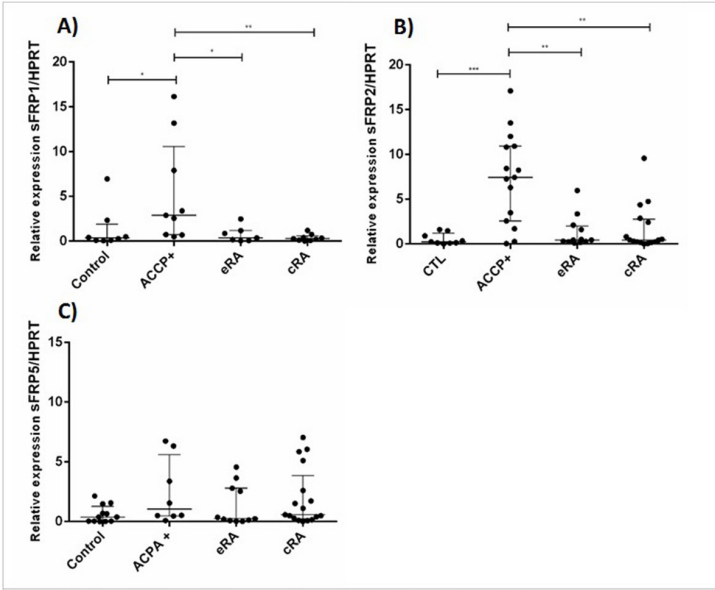
Age and gender distributions were similar between patients and controls, except for the group of cRA. Biochemical examination showed that serum levels of Anti-CarP were similar between all groups whereas the levels of ACCP autoantibodies were significant higher in all RA groups (ACPA+, eRA and cRA) when compared to healthy controls [$p < 0.05$; 16.98 vs 30.89, 345.1.30, 670.9] (Table 1).

Upregulation of sFRP2 and sFRP5 in ACCP+ Subjects

The expression levels of sFRP-3 and 4 is increased in activated PBMC from RA patients. We thus examined the expression levels of sFRP-1, sFRP-2 and sFRP-5 in RA patients and in high risk subjects (ACPA+) RT-qPCR assays show higher sFRP-1 levels in ACCP+ subjects than in healthy controls, eRA and cRA groups (figure 1A). Likewise,

Table 1: Demographics and serologicalparameters of the study population.

	Healthy controls	Relatives of subjects with ACCP positive	Early RA	Chronic RA
Age Mead (±SO)	43.0 ±14.52	36.88 ±10.94	38.8 0 ±12.01	54.82 ±10.02 *
Gender (F/M)	14/07	13/04	13/02	18/04
Values of ACCP Median (±SO).	16.98 ±5.71 (19/21)	30.89 ±4.96* (17/ 17)	345.1.3 0±638.1* (14/15)	670.9 ±602.1* (22/ 22)
Values of Anti Carb –P Median (±SD).	188.5 ±110.3 (10/21)	153.6 ±60.68 (17/ 17)	195.1 ±100.7 (9/15)	321.6 ±421.6 (20/ 22)



Differential expression of sFRP-l, 2, and 5 genes detected by semiquantitative RT-PCR analysis. **A,B** Predominant expression of sFRp-1 and 2 genes in subjects ACCP+. *p≤ 0.05, **p≤0.0l.

sFRP-2 levels were elevated in the ACCP+ group in comparison to the rest (Figure 1B). However, the expression levels of sFRP-5 were similar in all groups (Figure 1C).

Correlation Analysis

To determine any correlation between the expression levels of the Wnt regulators and the levels of ACPA or anti-CarP antibodies, we performed a non-parametric Spearman correlation analysis. Although, we did not find any correlation between regulators and autoantibodies, a positive correlatión was found between the expression of sFRP-5 and sFRP-1 when the groups of RA and ACCP+ were analyzed ($p < 0.05$; r = 0.452) (Table 2).

Discussion: sFRPs are described as modulators of the Wnt/Fzd signal transduction. sFRPs share the Wnt binding domain of the FZD receptors, but lack their 7-transmembrane segment. However, the pathophysiological functions of these proteins are not clear. Additionally, it is difficult to match proper Wnt/FZD ligand-receptor pairs because some Wnt proteins can interact with multiple FZD receptors.

Table 2: Correlation coefficients of the some clinical and relative expression.

		sFRP1	sFRP2	sFRP5	ACCP	AntiCarP
sFRP1	Correlation Coefficient	1	.028	.452*	-.231	-.132
			.901	.018	.247	.512
	N	27	22	27	27	27
sFRP2	Correlation Coefficient		1	.190	-.159	.017
				.275	.292	.919
	N		47	35	46	39
sFRP5	Correlation Coefficient			1	-.0 14	-.109
					.932	.520
	N			42	41	37
ACCP	Correlation Coefficient				1	-.046
						.761
	N				53	46
AntiCarP	Correlation Coefficient					1
	N					46

*.p≤0 ,05

In this study, we show for the first time the expression of sFRP1 and sFRP2 in high-risk subjects (6). Moreover, we also show that ACPA+ subjects have a higher expression of sFRP1 and sFRP2 in comparison to control subjects, eRA and cRA. RA patients typically have low bone mass at the start of the disease, indicating that bone damage already occurs before clinical inflammation (7, 8). Hence, recent RA onset should not exhibit signs of bone damage if inflammation is the sole contributor to bone loss. However, as mentioned, antibodies precede the onset of disease by several years, and thus represent an interesting alternative explanation for this finding (9, 10). More importantly, subjects with ACPA autoantibodies but no clinical signs of arthritis, show bone changes in the absence of inflammation (11). Considering the last and our results, we are led to suppose that this may be to the result of a compensatory process generated during the stages preceding the development of the disease, as in the increase in insulin levels during the stages prior to the diagnosis of diabetes mellitus. Our results are also in line with the roles of sFRP1, since it has been shown that this regulator is essential for proper development and maintenance of the joints (12). Therefore, it is reasonable that, being from the same family, sFRP-1 and sFRP-5 fulfill similar functions and therefore show a correlation between them.

REFERENCES

1. Boissier MC. Cell and cytokine imbalances in rheumatoid synovitis. Joint Bone Spine. 2011;78(3):230-4.
2. Boissier MC, Semerano L, Challal S, Saidenberg-Kermanac'h N, Falgarone G. Rheumatoid arthritis: from autoimmunity to synovitis and joint destruction. J Autoimmun. 2012;39(3):222-8.
3. Smolen JS, Steiner G. Therapeutic strategies for rheumatoid arthritis. Nat Rev Drug Discov. 2003;2(6):473-88.
4. Cruciat CM, Niehrs C. Secreted and transmembrane wnt inhibitors and activators. Cold Spring Harb Perspect Biol. 2013;5(3):a015081.
5. Imai K, Morikawa M, D'Armiento J, Matsumoto H, Komiya K, Okada Y. Differential expression of WNTs and FRPs in the synovium of rheumatoid arthritis and osteoarthritis. Biochem Biophys Res Commun. 2006;345(4):1615-20.
6. van de Stadt LA, van der Horst AR, de Koning MH, Bos WH, Wolbink GJ, van de Stadt RJ, et al. The extent of the anti-citrullinated protein antibody repertoire is associated with arthritis development in patients with seropositive arthralgia. Ann Rheum Dis. 2011;70(1):128-33.
7. Guler-Yuksel M, Bijsterbosch J, Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Ronday HK, Peeters AJ, et al. Bone mineral density in patients with recently diagnosed, active rheumatoid arthritis. Ann Rheum Dis. 2007;66(11):1508-12.
8. de Rooy DP, Kalvesten J, Huizinga TW, van der Helm-van Mil AH. Loss of metacarpal bone density predicts RA development in recent-onset arthritis. Rheumatology (Oxford). 2012;51(6):1037-41.
9. Syversen SW, Goll GL, van der Heijde D, Landewe R, Lie BA, Odegard S, et al. Prediction of radiographic progression in rheumatoid arthritis and the role of antibodies against mutated citrullinated vimentin: results from a 10-year prospective study. Ann Rheum Dis. 2010;69(2):345-51.
10. Nielen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum. 2004;50(2):380-6.

11. Kleyer A, Finzel S, Rech J, Manger B, Krieter M, Faustini F, et al. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. Ann Rheum Dis. 2014;73(5):854-60.
12. Thysen S, Luyten FP, Lories RJ. Loss of Frzb and Sfrp1 differentially affects joint homeostasis in instability-induced osteoarthritis. Osteoarthritis Cartilage. 2015;23(2):275-9.

Early recruitment of monocytes and granulocytes by excreted/secreted antigens of Taenia crassiceps

Guillermo Alvarado Hernandez, Yadira Ledesma-Soto, Luis Ignacio Terrazas Valdés

Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla de Baz, Mexico
galvaradohernandez@gmail.com; lesy_790413@yahoo.com.mx; literrazas@unam.mx

Keywords: PDL-1, MDSCs, STAT-1, TCES, STAT-6

Helminths are parasitic worms which comprise a class of pathogens that cause several diseases throughout the world. The immune response of mammals against these worms is complex. A model of infection by helminths is the cestode Taenia crassiceps. Its intact glycans and certain fractions of the in vitro-excreted/secreted (TcES) products, have demonstrated their capacity as adjuvants in the development of a TH2-type immune response, through regulation of maturation and activation of dendritic cells and macrophages. It has also been described that soluble Antigen from Taenia crassiceps has the ability to mobilize immature cells of myeloid origin with suppressive capacity (MDSCs). The MDSCs play a relevant role in the link between the innate and adaptive immune responses however; there are few investigations on parasitic infections by helminths where these cells were analyzed. In this work we evaluated the recruitment of granulocytes and monocytes induced after TcES inoculation TcEs for 48 h. We found that a population of neutrophils was recruited during the first 6 h after the inoculation. Soon after inoculation, two subpopulations of monocytes were recruited. We observed that while the percentage of monocytes increased until 48 h, both subpopulations of neutrophils were decreased significantly at the same time point. Interestingly, we found that both, neutrophils and monocytes expressed PDL-1 for short times. The ability of such in suppressing proliferation of T-lymphocytes stimulated with αCD3 was demonstrated. Then, these three subpopulations identified were separated by cell sorting. In the functionality test we found that Ly6Clow monocytes were more suppressive than Ly6Chi monocytes and Ly6G +Ly6C + neutrophils. However, the greatest suppressive effect occurred when the three populations acted synergistically. On the other hand, we show that the recruitment effect of Ly6Chi monocytes is dependent on STAT-1 and independent of a TH2 response by STAT-6 or IL-4Rα. Furthermore, we found that the absence of STAT1 significantly increased the recruitment of neutrophils in the peritoneum. Taking altogether, we concluded that: 1) the TcES have the capacity to recruit different cell populations (monocytes and neutrophils), 2) induce the early expression of suppressor molecules such as PDL1 and thus, 3) modulate the immune response

by suppressing the proliferation of T- lymphocytes. 4), STAT-1 is the main signaling pathway involved in the regulation of the recruitment of monocytes and neutrophils by TcES. 5) The expression of PDL1 in CD11b+ cells was dependent on STAT-1 and STAT-6. This study will help us to understand the mechanisms by which antigens such as the TcES, can modulate the inflammatory response in different diseases.

Effect of Taenia solium calreticulin anti-inflammatory on murine macrophages

Mayra Cruz-Rivera[1], Jose Alfredo Diaz-Gandarilla[2], Bernardo Oldak[3], Mariana Chavez[1], Ana Flisser[1], Fela Mendlovic[1,3]

[1]National Autonomous University of Mexico, Cuernavaca, Mexico
[2]Universidad Juárez Autónoma de Tabasco, Villahermosa, Mexico
[3]Universidad Anáhuac México Norte, Huixquilucan de Degollado, Mexico
mayracr@yahoo.com; digan123@hotmail.com; beroldak@gmail.com; 517chavez.vargas@gmail.com; flisser@unam.mx; fmendlo@yahoo.com

Keywords: Calreticulin, Helminths, Macrophages, Taenia solium, immuneregulation

Background: Macrophages are a heterogeneous population comprising different cell subsets. Upon activation, macrophages can be divided into two functionally different phenotypes (M1 and M2). M1 macrophages are associated with a type 1 immune response and production of INF-γ, while M2 macrophages induced by IL-4 and IL-13 cytokines are characterized by expressing high levels of arginase-1, mannose receptor and scavenger receptor A (SR-A). Helminth antigens have been shown to induce a M2 phenotype and are able to elicit regulatory immune responses and tissue repair. Several helminth-derived proteins with anti-inflammatory activity have been reported, such as the recombinant Taenia solium calreticulin (rTsCRT). We recently showed that rTsCRT induced a Th2 response by increasing the gene expression of IL-4 and IL-10 cytokines and prevented inflammation in experimentally induced colitis. However, the participation of rTsCRT on macrophage activation and the putative receptor involved have not been investigated.

Objective: Here we study the role of rTsCRT on macrophage activation in vitro and its interaction with the SR-A.

Methods: A murine macrophage line (J774A.1) was incubated with rTsCRT at different concentrations. Macrophages were stimulated for 24 hours. Cell culture supernatants from experimental and control cultures were collected for cytokine determination by ELISA and the cells were harvested for flow cytometry, qRT-PCR or confocal analysis. The relative expression of IL-10, TGF-β and arginase-1 was performed using commercially available TaqMan assays. The relative quantification was normalized by the ΔΔCt method, using β-actin as a house keeping gene. Flow cytometric analyses were

performed using anti-MHCII, anti-CD86, anti-CD206 and anti-SR-A as markers of the M2 phenotype. Confocal analyses were carried out to identify the SR-A and its interaction with rTsCRT.

Results: Macrophages treated with rTsCRT induced both IL-10 and TGF-β anti-inflammatory cytokines, as well a reduction of TNF-α. The expression of arginase-1 mRNA was found to be significantly increased in cell cultures stimulated with rTsCRT. Control cell cultures exhibited basal Arg-1 levels throughout the experiment. The flow cytometry analysis showed that stimulation with rTsCRT induced an increase of MHC II CD86 and SR-A expression compared to non-stimulated macrophages. Unlike other cell membrane molecules, CD206 was not up-regulated upon stimulation with rTsCRT. Finally, to test the SR-A and rTsCRT interaction, we performed an immunofluorescence assay. Macrophages were cultured on a coverslip and stimulated with rTsCRT, the results showed that rTsCRT colocalized with the SR-A on the surface of treated macrophages.

Conclusions: These data suggest that rTsCRT induces a M2 phenotype on J774A.1 macrophages, characterized by the expression of anti-inflammatory cytokines and up-regulation of Arg-1, MHCII, CD86 and SR-A. The interaction of rTsCRT with the SR-A and the signaling pathways involved merit further studies.

The co-stimulation of Transferon® and low concentrations of LPS increases the phagocytic activity in THP1- macrophages

G de J Carballo-Uicab[1], Hugo Valencia-Martínez[1], Gabriela Mellado-Sánchez[1], Luis Vallejo-Castillo[1,3], Emilio Medina-Rivero[1], Rommel Chacón-Salinas[1,4], Marco Velasco-Velázquez[1], Lenin Pavón[4], Sergio Estrada-Parra[4], Sonia Mayra Pérez-Tapia[1,4]

[1]Unidad de Desarrollo e Investigación en Bioprocesos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico

[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico

[3]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico

[4]Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz (INPRFM), Mexico City, Mexico

gregorio_1988@hotmail.com; drapi.9@hotmail.com; melladosg@gmail.com; vallejo0476153@msn.com; vallejo0476153@msn.com; emrivero@yahoo.com.mx; rommelchacons@yahoo.com.mx; rommelchacons@yahoo.com.mx; marcovelasco@hotmail.com; lkuriaki@gmail.com; sestradap07@hotmail.com; cipft.encb@gmail.com; cipft.encb@gmail.com; cipft.encb@gmail.com

Keywords: Phagocytosis, THP-1, immunomodulator, Transferon, Peptide derived drugs

Clinical studies have evinced the immunomodulatory properties of Transferon™, a peptide-derived biotherapeutic which has been authorized for human use by Mexican health authorities, however its specific cellular and molecular mechanisms still need to be detailed. Previously, our group studied the effect of Transferon™, alone or with LPS, on human macrophages (THP-1 cell line), to obtain basic knowledge about phagocytes as a cellular key target of this product. In that study, some pro and anti-inflammatory cytokines levels and surface markers such as co-stimulation molecules and MHC-class II were measured to characterize the effect of Transferon™ on this cell line. To complete this characterization, in the present study, the concomitant effect of LPS and Transferon™ on phagocytic activity of macrophages was evaluated using the same cell line. THP-1 monocytes were differentiated to macrophages using a PMA stimulus (50 ng/mL). Then, THP-1 macrophages were stimulated with high dose (1 μg/mL) or low dose (0.1 μg/mL) of LPS and three concentrations of Transferon™ (0.1, 1.0 and 10 μg/mL). Phagocytic function was measured by flow cytometry using pHrodo™ Red bioparticles conjugates assay; the increment of the phagocytic activity was measured as the increase of the median fluorescent intensity exhibited by THP-1 macrophages as consequence of bacteria engulfment. Preliminary results showed that at high

dose of LPS, none of the different concentrations of Transferon™ showed a change of phagocytic activity in comparison with LPS alone (1 μg/mL). Conversely, at low dose of LPS, the phagocytic activity of THP-1 macrophages stimulated with 10 μg/mL of Transferon™ exhibited a statistically significant increment ($p < 0.05$) respect to the control of LPS alone (0.1 μg/mL). Co-stimulation with 0.1 and 1.0 μg/mL of Transferon™ with low dose LPS induced a slight non-statistically significant increment on the phagocytic activity. These observations were reproducible in three batches of Transferon™ (17D11, 17D12 y 17D13). These preliminary **results** suggest that, in an appropriate co-stimulation condition with LPS, Transferon™ is able to enhance the phagocytic capability of macrophages. Further studies either in vitro or in vivo are needed to evince the mechanisms of action of Transferon™ during an infection.

The immunoactivation mechanism of Cry1Ac Bacillus thuringiensis protoxin in macrophages: Possible role of Vimentin as an interacting protein

Leticia Moreno Fierros, Damaris Ilhuicatzi Alvarado, Nestor Rubio Infante

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
draleticiamorenofierros@yahoo.com; ilhuicatzidamaris@hotmail.com; nes_3795@hotmail.com

Keywords: Interaction, Vimentin as an interacting protein, Pcry interaction with vimentin, Pcry and vimentin in activation of macrophages, activation macrophages

Mucosal surfaces are highly vulnerable to invasion of pathogens, and it is difficult to achieve significant immune responses at these mucosal sites, because they are highly regulated. One strategy to improve vaccines is the use of adjuvants but there are not still safe mucosal adjuvants available. We have proposed that Cry1Ac proteins from Bacillus thuringiensis, protoxin (pCry1Ac), may function as good mucosal adjuvant candidate, because particularly pCry1Ac is a potent systemic and mucosal immunogen and adjuvant able to increase protection against several infection models. The action mechanism of Cry1Ac protoxin seems to be mediated by its ability to activate antigen presenting cells such as macrophages by increasing the expression of costimulatory molecules and cytokine production. In an effort to explain the adjuvant mechanism of Cry1Ac protoxin we proposed the existence of possible receptors in mammals. Indeed, in a recent report we have demonstrated that macrophage activation induced by Bacillus thuringiensis Cry1Ac protoxin involves MAPK activation and the interaction with cell-surface-HSP70, furthermore we proposed vimentin as an additional binding protein for Cry1Ac protoxin. However, the interaction of vimentin with protoxin and the role of the toxic core of the molecule (the toxin), in the binding have not been determined.

Objective: Identify the binding proteins for Cry1Ac protoxin in murine macrophages and determine the role of the toxin in the interaction between Cry1Ac protoxin and vimentin.

Methodology: Cry1Ac protoxin is derived from the recombinant strain of E. coli JM103, is solubilized and proteolytically processed into toxin. We used RAW 264.7 cells to performed binding assays with FITC-labelled Cry1Ac proteins (toxin and

protoxin) at 4°C. We performed at 37°C confocal assays to determine if the binding of toxin could be followed by internalization. In order to determine the toxin and protoxin binding proteins we performed immunoprecipitation assays with Cry1Ac proteins conjugated to sepharose; the identification of immunoprecipitated proteins was carried out by MALDI-TOF-TOF. Vimentin, HSP70, Galectin and Actin were determined as protoxin and toxin binding proteins and their identity was confirmed by western blot assays. For Cry1Ac binding proteins determination we performed Blot ligand assays and cross-immunoprecipitation assays with specific vimentin antibodies. In order to determine the role of the toxic core in the interaction between Cry1Ac protoxin and vimentin we performed blot ligand assays and immunoprecipitations with or without toxin as competitor.

Results: Binding of Cry1Ac protoxin and toxin to macrophages surface was specific and saturable, at 37°C, binding was followed by internalization as co-localization of toxin with endosome markers Rab5 and Rab7 shows. The same immunoprecipitated proteins for both Cry1Ac protoxin and toxin include: vimentin, Galectin and Actin as MALDI-TOF-TOF shows, the Cry1Ac toxin and protoxin binding proteins identity was confirmed by western blot assays. Binding of Cry1Ac proteins with Vimentin, was demonstrated by Blot ligand assays and by cross-immunoprecipitation assays with specific vimentin antibodies we confirmed the interaction between Cry1Ac proteins with vimentin. By competition assays with Cry1Ac toxin we inhibited the binding of Cry1Ac protoxin to Vimentin.

Conclusions: Cry1Ac proteins bind to macrophages surface in a dose dependent way that can be mediated by a receptor. Cry1Ac toxin and protoxin bind to macrophages proteins: vimentin, Galectin and Actin. The toxic core plays a key role in vimentin-Protoxin interaction since the vimentin interacts mainly with this domain.

Monocytes and Th2 cells, possible mediators of the inflammatory and neurodegenerative response in untreated Parkinson disease patients

Jaquelin Leyva-Hernández, Laura Adalid-Peralta, Asiel Arce-Sillas, Diana Denisse Álvarez- Luquín, Ulises Rodríguez-Ortiz, Edda Lydia Sciutto- Conde, Gladis del Carmen Fragoso Gonzalez, Marie Catherine Boll- Woehrlen, Esteban Montes-Moratilla, Viridiana Vivas- Almazán, Citzielli Aseret Pérez

Instituto Nacional de Neurología y Neurocirugía (INNN), Mexico City, Mexico
jackley91@hotmail.com; adalid.laura@yahoo.com; dobleas_2005@hotmail.com; danliuc@hotmail.com; ulrdz@me.com; argentina54@hotmail.com; gladisfragoso@hotmail.com; bollneur@gmail.com; esteban22ntdv@hotmail.com; virydianaamane@gmail.com; citziaseret@hotmail.com

Keywords: Dopaminergic Neurons, Inflammation, Monocytes, Parkinson Disease, cells Th2

Introduction: A degeneration of dopaminergic neurons in the substantia nigra and α-synuclein accumulations are the hallmark of Parkinson disease (PD). Activation of glial cells, production of ROS and proinflammatory cytokines, and infiltration of peripheral cells are also observed. The regulatory populations that participate in PD are not known, and the role of proinflammatory populations in the disease is controversial.

Objective: To characterize the regulatory and proinflammatory cell populations in untreated EP patients.

Material and Methods: Regulatory (Tregs, Bregs, CD8regs, dendritic cells, and monocytes) and proinflammatory (Th1, Th2, Th17, dendritic cells, and monocytes) cell populations were characterized by flow cytometry in PBMCs from 10 controls and 15 untreated PD patients.

Results: The levels of IL-12-producing classical monocytes, IL-4-producing Th2 cells, IL-13-producing Th2 cells, and TCD8 regulatory cells were increased in PD patients. On the other hand, activated Treg levels were decreased in PD patients. A positive correlation between IL-4-producing Th2 cells and IL-13-producing Th2 cells was observed. Additionally, IL-12-producing classical monocytes correlated with HLA-DR-expressing monocytes, and classical monocytes correlated with IL-4-producing

Th2 cells. A negative correlation was observed between IL-12-producing classical monocytes and dendritic cells presenting SLAMF1, and also between IL-12-producing classical monocytes and Tregs.

Discussion and Conclusions: The decrease in peripheral Tregs, which suggests a deficit in immune regulation in EP, could explain the increase in T CD8Reg cells. Neuroinflammation could be mediated by IL-12-producing classical monocytes and IL-13- and IL-4-producing Th2 cells. A relation between the presence of receptors to these cytokines and dopaminergic neuron death was described recently; thus, the peripheral increase of IL-13 and IL-4 could play a role in neurodegeneration in PD.

Role of interferon (IFN)-gamma over the induction of T regulatory lymphocytes in experimental autoimmune encephalomyelitis

Gabriel Arellano[1], Cristian Castillo[1], Eric Acuña[1], Stephen D. Miller[2], Rodrigo Naves[1]

[1]*Universidad de Chile, Santiago, Chile*
[2]*Feinberg School of Medicine, Northwestern University, Chicago, United States*
g.arellanolorca@gmail.com; cmarcelocb@gmail.com; hanamvericta@gmail.com; s-d-miller@northwestern.edu; rnaves@gmail.com

Keywords: Multiple Sclerosis, EAE, T regulatory cells, IFN-gamma, TGF-beta, PD-L1

Introduction: Multiple sclerosis (MS) is a chronic autoimmune disease affecting the central nervous system (CNS), characterized by a lost of immune tolerance, that includes a functional inactivation of regulatory T lymphocytes (Tregs). Recent studies have described different mechanisms by which Interferon (IFN)-gamma, known classically as a pro-inflammatory cytokine, has regulatory and protective effects in the animal model of MS, the Experimental Autoimmune Encephalomyelitis (EAE). Previous results of our laboratory have shown that in vivo treatment with IFN-gamma during the chronic phase of EAE induced a significant amelioration of symptoms, associated with a significant increase of Tregs in the SNC of these mice. In this study, we analyzed the mechanisms by which IFN-gamma induced the increase of Tregs suppressing EAE.

Results: The results obtained shown that depletion of cells expressing FOXP3 suppressed the protective effects of IFN-gamma, supporting the notion that the therapeutic effect of this cytokine is dependent on Tregs. Assays performed in vitro with CD4+CD25- T lymphocytes obtained from EAE mice revealed that IFN-gamma did not exert a direct effect on the induction or functionality of Tregs. In addition, IFN-gamma inhibited FOXP3 expression in conditions of iTregs differentiation (TGF-beta + IL-2). Likewise, IFN-gamma did not affect the expression of regulatory molecules, FOXP3 expression, or functionality of Tregs obtained from EAE mice. The in vivo combined administration of IFN-gamma and neutralizing antibodies against IL-10, TGF-beta or PD-1, showed that the protective effect of IFN-gamma in the chronic phase of EAE depends on TGF-beta and PD-1/PD-Ls pathway, but not on IL-10. Analysis of regulatory molecules expression in different cell subpopulations obtained from EAE mice determined that IFN-gamma induced an increased expression of TGF-beta and PD-L1 on different subgroups of CD11b+ cells (antigen presenting cells) and TGF-beta on CD8+ T cells. In addition, it was found that both in vivo and in vitro, IFN-gamma

–stimulated CD11b+ cells were able to induce in a TGF-beta and PD-L1-dependent manner an increase of Tregs frequency starting from naïve T CD4+ cells obtained from healthy (without EAE) wild type or 2D2 (expressing a specific TCR for myelin peptide) mice.

Conclusion: Overall, these results reveal that the beneficial effects of IFN-gamma in the chronic phase of EAE are related with an increase of Tregs in the SNC mediated by the expression of FOXP3, TGF-beta and PD-1/PD-Ls pathway, and associated with an enhance in the expression of TGF-beta and PD-L1 on CD11b+ cells and TGF-beta on T CD8+ cells.

Chronic high Fructose ingestion in water decrease miR-155-5p and increase SOCS1 and C/EBPB expression in rat visceral adipose tissue

Javier González-Ramírez[1], Adrián Hernández-Díazcouder[2], Santiago Villafaña[3], Rodrigo Romero-Nava[3], Armando Ruiz-Hernández[3], Julio Cesar Almanza-Pérez[4], Erika Contreras-Núñez[4], Paloma Karina Barrera-Reyes[5], Fausto Sánchez[4], Rafael Bojalil[2], Fausto Sanchez-Munoz[2]

[1]Universidad Autónoma de Baja California, Mexicali, Mexico
[2]Instituto Nacional de Cardiología, Mexico City, Mexico
[3]Instituto Politécnico Nacional, Mexico City, Mexico
[4]Universidad Autónoma Metropolitana, Mexico City, Mexico
[5]National Institute of Genomic Medicine, Mexico City, Mexico
javier.gonzalez.ramirez@uabc.edu.mx; couder_hell@hotmail.com; svillafana@ipn.mx; roloromer@gmail.com; armandoruizhernandez@gmail.com; julcesarmac2@yahoo.com.mx; bioexp_econe@live.com.mx; pbarrera@inmegen.gob.mx; sygf2122@correo.xoc.uam.mx; rafaelbojalil@gmail.com; fausto22@yahoo.com

Keywords: Adipogenesis, Adipose Tissue, Fructose, IL6, TNF-α, SOCS-1, C/EBP-β, miR-155-5p

Background: Fructose consumption increases lipogenesis by regulating gene expression programs related to adipose tissue expansion and adipocyte differentiation. To characterize these processes, we determined gene expressions of miR-155-5p, TNF-α, SOCS-1 and C/EBP-β in visceral adipose tissue in rats consuming high concentrations of fructose in water.

Results: Fructose group showed higher fat pad mass, systolic blood pressure and plasma triglycerides but lower HDL-Cholesterol ($P < 0.05$) as compared to controls. miR-155-5p expression was reduced in the Fructose group ($P < 0.05$). No differences were observed in mRNA levels of Socs1 and Cebpb, while, the protein expression of this genes in the Fructose group were increased two folds as compared to the Control group ($P < 0.05$). Il6 mRNA expression was increased almost ten times in the Fructose group, whereas, Tnf expression in controls was two folds higher than in the Fructose group ($P < 0.05$).

Conclusions: High fructose consumption, other than adipose tissue expansion, induce decreased Tnf and miR-155-5p and increased C/EBP-β and SOCS-1 protein expression.

Regulatory T cell induction by excretion-secretion products from taenia crassiceps cysticerci for possible antiinflammatory use

Laura Adalid-Peralta[1], Valeria Morales-Ruiz[1], Dina López-Recinos[1], Adrián Bernardo Guevara-Salinas[1], Sandra Gómez-Fuentes[1], Cristina Parada-Colín[2], Clara Inés Espitia-Pinzón[2], Marisela Hernández-González[2], Silvia Ivonne Mora-Herrera[2], Gladis Del Carmen Fragoso-González[2], Edda Lydia Sciutto-Conde[2]

[1]Instituto Nacional de Neurología y Neurocirugía (INNN), Mexico City, Mexico
[2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
adalid.laura@yahoo.com; fugas_033_koala@hotmail.com; chinita_2_89@hotmail.com; adriandtonic@gmail.com; sammxmx@hotmail.com; cristinaparada2004@yahoo.com.mx; espitia@biomedicas.unam.mx; marysela2004@yahoo.com.mx; sivonnemor@biomedicas.unam.mx; gladis@unam.mx; argentina54@hotmail.com

Keywords: Cysticercosis, regulatory T cells, Parasitic infections, Taenia crassiceps, excretion-secretion products

Introduction: The role of T regulatory cells (Tregs) is related to inflammation control in several parasitic infections, allowing the parasite to establish in the host. T. crassiceps cysticerci can modulate the immune response by several mechanisms, like molecule excretion and secretion into the environment. Some helminth excretion-secretion proteins (ESP) create an immunoregulatory environment. However, the characteristics of these molecules are not known. This project intends to identify Treg-inducer cysticercal ESP.

Material and Methods: ESP were obtained from six independent T. crassiceps cysticercus cultures. These products were intraperitoneally inoculated to BAlb/c mice at different doses. Five days later, peritoneal cells were obtained, and Treg population was assessed by flow cytometry. Treg-inducer and non-Treg-inducer products were desalted and quantified. The quality of all extracts was evaluated by one-dimension SDS-PAGE. The extracts were concentrated with TCA and resolved in two-dimension gels (IEF pH 3-10 y 7158 V/6h).

Results: Among the ESP obtained, four induced Tregs and two failed to induce Tregs. One ESP from each group was analyzed by two-dimension gels and its peptide fingerprint was characterized with the ImageMaster-Platinum software. Protein profile

for each ESP showed differential spots between Treg-inducer and non-Treg-inducer products.

Discussion: Those ESP selected as inducer caused a significant Treg increase at a dose of 250 µg. The protein profile showed 34 differential spots in the inducer ESP, among which sequencing candidates were selected.

Conclusion: The sequencing and expression of these molecules will allow us to evaluate their Treg-inducer capacity in vivo and in vitro.

The HIV-1 envelope induces fusion between monocytes and lymphocytes generating hybrid cells with an activated monocyte-like phenotype mediated by intracellular TL2/TLR4 signalling

David Martínez Méndez, Evelyn Rivera Toledo, Ileana Licona Limon, Enrique Ortega Soto, Leonor Huerta Hernández

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
davidmm@iibiomedicas.unam.mx; evelynmicro@gmail.com; pantagato@gmail.com; ortsoto@biomedicas.unam.mx; leonorhh@biomedicas.unam.mx

Keywords: Monocytes, immunology, Fusion, gp120, HIV-1 Infection, Limphocytes

Background: Enveloped viruses induce cell-cell fusion when infected cells expressing viral envelope proteins interact with cells expressing the CD4 and coreceptor molecules, or through the contact of cell-free viral particles with adjoining target cells [1,2]. Fused cells are frequently observed in cultures of human leukocytes infected with HIV-1 isolates from patients with HIV-1 infection [3]. In vivo, multinucleated cells expressing viral antigens are a hallmark of HIV-1 and SIV infections in the nervous system, and the presence of these cells is associated with severe neuropathology [4,5]. Likewise, infected multinucleated cells have also been observed in lymph nodes of asymptomatic HIV-1 infected individuals [6,13]. Expression of myeloid markers indicates that multinucleated cells may originate from dendritic cells [7,9,12] or macrophages [4–14] in these tissues. We have previously reported that lymphoid Jurkat T cells expressing the HIV-1 envelope protein (Env) can fuse with THP-1 monocytic cells, forming heterokaryons with a predominantly myeloid phenotype [15, 16]. The implications of fusion between lymphocytic and monocytic cells in HIV pathogenesis are not well understood and may produce heterokaryons with an activated monocyte-like phenotype with important immunological consequences.

Objective: We determined the effect of fusion between lymphoid cells expressing the HIV-1 envelope protein (Jurkat Env+) and monocytic (THP-1) cells, on the expression of myeloid and lymphoid markers, phagocytic activity and the activation state. Since hybrids, but not cocultured unfused cells, showed a morphology resembling activated monocytes in the absence of differentiation-induced agents we also explored the mechanism involved in this case of differentiation induced by fusion.

Material and Methods: Fusion partners were differentially stained with the red fluorescent DiI and green fluorescent DiO lipophilic dyes. Fusion was induced by the coculture of DIO-Env+ Jurkat and DiI-THP-1 cells at a 1:1 ratio in a 48- flat bottom well plates. For the fusion assays, DiO-labeled Env+ Jurkat cells were pre incubated for 30 min with 60 nM of the HIV fusion inhibitor peptide T-20 before addition of DiI-THP-1 cells. Cells were collected from plates, washed, and incubated with APC conjugated monoclonal antibodies against the CD3, CD28, CD32, or CD68 antigens and the expression of markers on double (hybrids) and single (unfused) fluorescent cells was analyzed 24 hours later by flow cytometry. Phagocytic activity was determined by mixing of cocultured cells with opsonized erythrocytes labeled with APC, at a cell:erythrocyte ratio of 1:20 at 37 °C for 1 h. As controls, identical samples were incubated at 4°C and in the presence of Cytochalasin D. For inhibition of the TL2/TL4 signaling pathway, DiO-Env+ Jurkat and DiI-THP-1 cells were pre-incubated separately with 5 or 15μM of the TIRAP-inhibitor NBP2-26245, or with the antennapedia control peptide (20 μM) for 1 hour at 37^{0}C with 5% CO2. Percentage of fusion and expression of the differentiation marker CD11b was determined after additional 24 h of coculture.

Results: Heterokaryons showed a stable expression of monocytic markers CD32 and CD68, whereas the expression of lymphoid markers CD3 and CD28 was mostly lost. Like THP-1 cells, 50% of hybridss exhibited FcγR-dependent phagocytic activity and showed an enhanced expression of the activation marker ICAM-1 upon stimulation with PMA. In addition, 54% of hybrids showed morphological changes compatible with maturation, as well as high expression of the differentiation marker CD11b in the absence of differentiation-inducing agents. No morphological change, nor increase in CD11b expression were observed when an HIV-fusion inhibitor blocked fusion, or when THP-1 cells were cocultured with Jurkat cells expressing a non-fusogenic Env protein, showing that differentiation was not induced merely by cell-cell interaction but required cell-cell fusion. Inhibition of TLR2/TLR4 signaling by a TIRAP inhibitor greatly reduced the expression of CD11b in hybrids. Thus, lymphocyte-monocyte heterokaryons induced by HIV-1 Env are stable and functional, and fusion prompts a phenotype characteristic of activated monocytes via intracellular TLR2/TLR4 signaling.

Conclusions: In summary, HIV-1 Env protein mediates the fusion of lymphoid and monocytic cells, forming hybrid cells with a predominantly myeloid phenotype. Hybrids showed phagocytic activity similar to that of the parental monocytes. Fusion induces monocyte differentiation events that may be mediated by interaction of the HIV-1 Env precursor with intracellular TLR4 and/or TLR2, providing a mechanism of monocyte activation that could not be induced by interactions at the surface level.

Our observations implicate that fusion of monocytes with T cells through HIV Env may lie beneath the formation of multinucleated cells during HIV-1 infection.

REFERENCES

[1] F. Clavel, P. Charneau, Fusion from without directed by human immunodeficiency virus particles, J. Virol. 68 (1994) 1179–1185.

[2] J.D. Lifson, M.B. Feinberg, G.R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K.S. Steimer, E.G. Engleman, Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein, Nature 323 (1986)

[3] J.T. Kimata, L. Kuller, D.B. Anderson, P. Dailey, J. Overbaugh, Emerging cytopathic and antigenic simian immunodeficiency virus variants influence AIDS progression, Nat. Med. 5 (1999) 535–541.

[4] D.J. Eilbott, N. Peress, H. Burger, D. LaNeve, J. Orenstein, H.E. Gendelman, R. Seidman, B. Weiser, Human immunodeficiency virus type 1 in spinal cords of acquired immunodeficiency syndrome patients with myelopathy: expression and replication in macrophages, Proc. Natl. Acad. Sci. USA 86 (1989) 3337–3341.

[5] J.E. Bell, The neuropathology of adult HIV infection, Rev. Neurol. 154 (1998) 816–829.

[6] A. Rinfret, H. Latendresse, R. Lefebvre, G. St-Louis, P. Jolicoeur, L. Lamarre, Human immunodeficiency virus-infected multinucleated histiocytes in orophar- yngeal lymphoid tissues from two asymptomatic patients, Am. J. Pathol. 138 (1991) 421–426.

[7] S.S. Frankel, B.M. Wenig, A.P. Burke, P. Mannan, L.D. Thompson, S.L. Abbondanzo, A.M. Nelson, M. Pope, R.M. Steinman, Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid, Science 272 (1996) 115–117.

[8] J.L. Dargent, L. Lespagnard, A. Kornreich, P. Hermans, N. Clumeck, A. Verhest, HIV-associated multinucleated giant cells in lymphoid tissue of the Waldeyer's ring: a detailed study, Mod. Pathol. 13 (2000) 1293–1299.

[9] S.S. Frankel, K. Tenner-Racz, P. Racz, B.M. Wenig, C.H. Hansen, D. Heffner, A.M. Nelson, M. Pope, R.M. Steinman, Active replication of HIV-1 at the lymphoepithelial surface of the tonsil, Am. J. Pathol. 151 (1997) 89–96.

[10] S.B. Kapadia, C.A. Wiley, V. Soontornniyomkij, G. Wang, S.H. Swerdlow, HIV- associated Waldeyer's ring lymphoid hyperplasias: characterization of multinu- cleated giant cells and the role of Epstein-Barr virus, Hum. Pathol. 30 (1999) 1383–1388.

[11] J.M. Orenstein, S.M. Wahl, The macrophage origin of the HIV-expressing multi- nucleated giant cells in hyperplastic tonsils and adenoids, Ultra. Pathol. 23 (1999) 79–91.

[12] L. Bhoopat, T.S. Rithaporn, S. Khunamornpong, T. Bhoopat, C.R. Taylor, P.S. Thorner, Cell reservoirs in lymph nodes infected with HIV-1 subtype E differ from subtype B: identification by combined in situ polymerase chain reaction and immunohistochemistry, Mod. Pathol. 19 (2006) 255–263.

[13] M. Lewin-Smith, S.M. Wahl, J.M. Orenstein, Human immunodeficiency virus-rich multinucleated giant cells in the colon: a case report with transmission electron microscopy, immunohistochemistry, and in situ hybridization, Mod. Pathol. 12 (1999) 75–81.

[14] V. Soontornniyomkij, G. Wang, S.B. Kapadia, C.L. Achim, C.A. Wiley, Confocal microscopy assessment of lymphoid tissues with follicular hyperplasia from patients infected with human immunodeficiency virus type 1, Arch. Pathol. Lab Med. 122 (1998) 534–538.

[15] E. Rivera-Toledo, L. Huerta, C. Larralde, E. Lamoyi, Quantitative and phenotypic analyses of lymphocyte-monocyte heterokaryons induced by the HIV envelope proteins: significant loss of lymphoid markers, Exp. Mol. Pathol. 90 (2011) 157–166.

[16] Martínez-Méndez D, Rivera-Toledo E, Ortega E, Licona-Limón I, Huerta L. Monocyte-lymphocyte fusion induced by the HIV-1 envelope generates functional heterokaryons with an activated monocyte-like phenotype. Exp Cell Res. 2017 Mar 1;352(1):9-19. doi: 10.1016/j.yexcr.2017.01.014. Epub 2017 Jan 27.

In vitro analysis of Immunoregulatory capacities of skin derived mesenchymal stromal cells from healthy donors and patients with psoriasis

Víctor Adrián Cortés-Morales[1], Laura Cecilia Bonifaz-Alfonzo[1], Marta Elena Castro-Manrreza[2], Octavio Castro-Escamilla[1], Hector Mayani[1], Jessica Mayte Hernández-Cristino[1], Juan José Montesinos[1]

[1]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
v.adrian.cortes@gmail.com; labonifaz@yahoo.com; elmar_ca@yahoo.com.mx; larenmc@msn.com; hmayaniv@prodigy.net.mx; mesenquimales@yahoo.com.mx; montesinosster@gmail.com

Keywords: Mesenchymal Stromal Cells, Psoriasis, T cell regulation, Inmunomodulation, skin inmunoregulation

Introduction: Mesenchymal Stromal Cells (MSCs) have immunoregulatory properties against several immunological cells (Castro-Manrreza and Montesinos, 2015). MSCs affect T cell proliferation and differentiation, primarily through immunosuppressive molecules and generation of regulatory T cells (Castro-Manrreza et al, 2014). The main source of MSCs is Bone Marrow (BM), but some studies have proposed the presence of MSCs in dermis of healthy donors and patients with psoriasis (Orciani et al, 2011; Najar and Lagneaux, 2017). However, few studies have analyzed their effect against CD3+ T cells in a coculture system. Thus, determination of immunoregulatory capacities is very important to understand their participation in an inflammatory process like psoriasis.

Aim: To evaluate in vitro the immunoregulatory capacity of skin derived MSCs from healthy donors and patients with psoriasis against T cells.

Methods: Through cell migration system of skin fragments, MSCs were obtained from: dermis of psoriasis lesion (Pso-DL) and non-lesion (Pso-DNL). MSCs of dermis of healthy skin (C-Der) and BM (C-BM) were obtained as controls. MSCs were characterized for CD90, CD105, CD73, HLA-I, HLA-II, CD45, CD34, CD31 and CD14 by flow cytometry and their capacity for adipogenic, osteogenic and chondrogenic differentiation was evaluated. Peripheral blood mononuclear cells (PBMC) and CD3+ enrichment T cells from healthy donors were obtained through density gradient and were activated with phytohemagglutinin or anti-CD3/CD28 beads respectively. T cells were cocultivated in the absence or presence of MSCs. By flow cytometry were eval-

uated CD3+, CD4+ and CD8+ T cell proliferation, expression of CTLA-4 in T cells, generation of regulatory T cells, expression of programmed death-ligand 1 (PD-L1) in all MSCs and finally we evaluated concentration of interleukin 4 (IL-4), interleukin 10 (IL-10), tumor necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ) in conditioned medium.

Results: MSCs of Pso-DL, Pso-DNL and C-Der showed adipogenic, osteogenic and chondrogenic differentiation capacity indicated by oil red, alkaline phosphatase activity and chondrogenic matrix colored with Alcian blue in cryosections from pelleted micromasses, respectively. Also they expressed membrane markers (CD90, CD73 and CD105) and were negative for endothelial and hematopoietic markers (HLA-II, CD45, CD34, CD31 and CD14). This phenotype for MSCs was established by the International Society for Cellular Therapy (ISCT) (Dominici et al, 2006). It was observed that skin derived MSCs from patients with psoriasis and healthy donors, did not have capacity to decrease T cell proliferation or to generate regulatory T cells, as compared with those from BM. Skin derived MSCs from patients with psoriasis and healthy donors, did not modify the expression of CTLA-4 in T cells and their coculture system showed a decreased level of IL-4, IL-10 and TNF-α as compared with BM, however we observed increased INF-γ levels. MSCs from the skin of patients with psoriasis and healthy donors showed increased PD-L1 expression in a coculture system.

Conclusions: The decreased immunosuppressive capacity of MSCs from skin may possibly contribute to the inflammatory microenvironment in this tissue.

REFERENCES

Castro-Manrreza ME et al. "Human Mesenchymal Stromal Cells from Adult and Neonatal Sources: A Comparative in Vitro Analysis of Their Immunosuppressive Properties against T Cells." Stem cells and development 23.11 (2014): 1217–32.

Castro-Manrreza ME and Montesinos JJ. "Immunoregulation by Mesenchymal Stem Cells : Biological Aspects and Clinical Applications." Journal of Inmunology Research 2015.394917 (2015).

Dominici M et al. "Minimal Criteria for Defining Multipotent Mesenchymal Stromal Cells. The International Society for Cellular Therapy Position Statement." Cytotherapy 8.4 (2006): 315–7.

Najar M and Lagneaux L. "Foreskin as a Source of Immunotherapeutic Mesenchymal Stromal Cells." Immunotherapy 9.2 (2017): 207–217.

Orciani M et al. "The Mesenchymal Stem Cell Profile in Psoriasis." British Journal of Dermatology 165.3 (2011): 585–592.

NR4A2 and NR4A3 expression differ in immune cells from RSA patients compared to normal fertile women

Carlos De La Torre Hernandez, Erika Rudi, Josefina Stagnaro, Eloisa Irene Arana, Pablo Mariano Fernandez

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
dr.delatorre@gmail.com; rudi.eriika@gmail.com; josefinastagnaro@hotmail.com; eloarana@yahoo.com; pfernandez1120@gmail.com

Keywords: protein expression, mRNA expression, Immune Regulation, spontaneous abortion, NR4A orphan receptors

Background: Spontaneous abortion is the most common complication of early pregnancy. Recurrent Spontaneous Abortion (RSA) is defined as three consecutive losses within the first 20 weeks of gestation. The majority of these recurrent losses remain unexplained. NR4A orphan receptors function as ligand-independent transcription factors that regulate the expression of target genes. These receptors have been implicated in broader functions within the immune system cells. NR4A receptors may play a role in restraining or increasing the effects of immune system cells in embryo development and implantation.

Methods: Peripheral blood mononuclear cells (PBMCs) were obtained from RSA patients and control groups. NR4A mRNA expression was determined by real-time PCR. NR4A protein expression was determined by flow cytometry. Statistical analysis was performed using GraphPad Prism 5 software. Groups were compared by Mann-Whitney test.

Results: Samples from PBMCs in RSA group showed significant lower mRNA expression levels of NR4A2 (p=0.0174) and NR4A3 (p=0.027) nuclear orphan receptors, when compared to normal fertile women. NR4A2 protein expression was significantly higher in PBMCs from RSA patients when compared to normal fertile women (p=0.0014) and this was also observed when analyzing independent immune cells subsets CD3+CD4+ (p=0.0025), CD3+CD8+ (p=0.0015), CD3-CD56+ (p=0.002) and CD3-CD14+ (p=0.002). Similar significant higher protein expression levels were observed for NR4A3 in PBMCs (p=0.0066), or CD3+CD4+ (p=0.0066), CD3+CD8+ (p=0.0088), CD3-CD56+ (p=0.0106) and CD3-CD14+ (p=0.0118) immune cells sub-

sets. NR4A1 mRNA or protein expression showed no significant difference between the analyzed groups.

Conclusion: These results strongly support our hypothesis that NR4A orphan nuclear receptors play a role in the immune regulation mechanisms contributing to a successful pregnancy, and point to NR4A2 and NR4A3 as the main NR4A subfamily receptors that would participate as immune restrictive factors for embryo implantation or development.

Epigenetic changes (miR-155 expression levels) in immune cells in mexican women exposed to polycyclic aromatic hydrocarbons

Iván Nelinho Pérez-Maldonado, Tania Ruiz-Vera, Angeles Catalina Ochoa-Martinez

Facultad de Medicina, Universidad Autónoma de San Luis Potosi, San Luis Potosí, Mexico
ivan.perez@uaslp.mx; truizv@live.com.mx; miaka_naeguino@hotmail.com

Keywords: immune cells, miR-155, PAHs, Cardivascular diseases, epigenetic mechanims

Introduction: The domestic use of solid fuels continues to be a major problem worldwide. In Mexico, 22.5 million people use wood as their main energy source. These materials are usually burned in rustic inefficient systems, leading to the formation of several toxic compounds. Among those, polycyclic aromatic hydrocarbons (PAHs) are generated as a mixture that can vary in its composition according to several factors like type of material burned (Pruneda-Alvarez et al., 2016). Recent studies suggest that PAHs exposure could be an additional factor for impaired vascular health and atherogenesis processes, events that gradually lead to cardiovascular diseases (CVD) (Pruneda-Alvarez et al., 2016). However, the underlying molecular mechanism for which PAHs induced adverse effects on the cardiovascular system have been not elucidated. At this respect, it has been demonstrated that immune cells (such as monocytes, macrophages and T cells) are key players in the development of that kind of diseases (CVD). Moreover, it is becoming progressively clear that epigenetic changes administer numerous characteristics of immune cells. Then, regulation of epigenetic alterations offers chances to modify disease-associated epigenetic states. Gene promoters methylation, histones acetylation and mRNA transcripts silencing by noncoding RNAs (ncRNAs) are including in the mechanisms of epigenetic alteration (Sierra et al., 2016). Specifically, miRNAs can regulate several biological characteristics, such as cell death, cell proliferation, differentiation, organ development and organ physiology (Sierra et al., 2016). Besides, it has been suggested that microRNAs may serve as medical therapeutic targets (Sierra et al., 2016). In this context, miR-155 is a ubiquitous multifunctional miRNA that has recently emerged as a novel component of inflammatory signal transduction in the pathogenesis of atherosclerosis (Wei et al., 2013). miR-155 can modulate the expression of genes correlated with inflammation in different cell types, playing a protective/anti-inflammatory role during foam cell formation in atherosclerosis (Li et al., 2016).

Objective: Therefore, the aim of this study was to evaluate the expression levels of miR-155 in immune cells from Mexican women exposed to PAHs.

Methods: A cross-sectional observational study was performed in a group of 100 women from San Luis Potosi State, Mexico. Women included in the research signed an informed consent of voluntary participation and was approved by the Bioethics Committee of the School of Medicine, at the Autonomous University of San Luis Potosi. Blood samples of each participant were obtained to perform clinical biochemistry analysis and to determine the expression of miR-155. Also, from blood samples were isolated immune cells (peripheral blood mononuclear cells) by Ficoll–Hypaque density-gradient centrifugation method (Sigma-Aldrich, St. Louis MO, USA). After isolation process, total RNA was extracted from cell pellet using TRIzol reagent in accordance with the manufacturer´s instructions. After, RNA was transcribed into cDNA using TaqMan® MicroRNA Reverse Transcription kit according to manufacturer´s instructions. Subsequently, 100 ng of cDNA was amplified using TaqMan® Universal Master Mix and TaqMan® MicroRNA Assay primers for human miR-155. Reactions were carried out in StepOne Real-Time PCR system. Data analysis of human miR-155 expression levels were accomplished using relative miRNA expression through the method 2-ΔΔCt normalized to U6 as an endogenous control. First-morning urine samples were collected to determine 1-OHP and creatinine levels. Urinary 1-OHP was quantified following the method described previously by our research group elsewhere (Pruneda-Alvarez et al., 2016). Anthropometric measurements, blood pressure, and clinical determinations were performed according to Pruneda-Alvarez et al. (2016). Regarding statistics, a descriptive data analysis (mean, standard deviation, minimum, maximum, frequencies, and distribution) was performed. Then, to assess the effects of PAHs exposure (1-OHP levels) on the outcome variable (miR-155 expression levels) multivariable linear regression analyses were performed by forward stepwise model selection, setting a significance level of 0.05 for entry and 0.10 for removal from the model, and the goodness of fit from the final model was analyzed by R2 statistics. All analyses were performed using JMP IN 7.0 software (SAS Institute, Inc., Cary, NC, USA).

Results and Discussion: A reference value for non-occupationally exposed to PAHs and non-smoking population has been proposed (0.24 μmol/mol Cr) (Jongeneelen, 2001). Table 1 (supplementary material) shows the clinical parameters of women grouped according to the urinary 1-OHP concentration as those "Low exposed" and those "High exposed", taking as cutoff the reference value level. Expression levels in immune cells for miR-155 was significantly greater in the women with 1-OHP levels above 0.24 μmol/mol creatinine compared to the women with lower 1-OHP levels (p=

Table 1. Clinical parameters of women grouped according to the reference level of 1-OHP.

Variable	Low exposed [a] (n= 34)	High exposed [b] (n= 66)	P value
Age (years) [c]	36 ± 14.5 (21.0 - 80.0)	44.2 ± 17.6 (19.0 - 81.0)	**0.0164**
BMI (kg/m^2) [c]	26.7 ± 4.2 (19.9 – 37.8)	26.7 ± 4.6 (19.9 - 40.3)	0.9731
% Normal (20 - 24.9)	30	36	
% Overweight (25 - 29.9)	43	39	
% Obesity (>30)	27	25	
SBP (mm Hg) [c]	117 ± 19.4 (90.0 – 160)	118 ± 19.7 (90.0 – 162)	0.8227
DBP (mm Hg) [c]	70.8 ± 14.5 (54.0 – 100)	66.1 ± 9.3 (50.0 - 90.0)	0.2200
% Hypertension [d]	18	15	
Glucose (mg/dL) [c]	81.8 ± 30.0 (56.6 – 433)	103 ± 47.2 (55.7 – 389)	**0.0125**
% > 100 mg/dL	13	29	
Triglycerides (mg/dL) [c]	149 ± 64.1 (59.7 – 426)	155 ± 89.3 (16.5 – 673)	0.7372
% > 150 mg/dL	48	57	
Total cholesterol (mg/dL) [e]	175 ± 32.0 (114 – 255)	184 ± 43.1 (98.5 – 286)	0.2757
% > 200 mg/dL	19	33	
HDLc (mg/dL) [c]	49.0 ± 15.9 (19.0 - 94.7)	51.9 ± 14.2 (33.1 – 144)	0.3872
% < 50 mg/dL	61	50	
LDLc (mg/dL) [e]	90.7 ± 30.1 (35.1 – 166)	103 ± 35.7 (26.4 – 180)	0.1062
% > 130 mg/dL	6	22	
AIP [c]	0.12 ± 0.25 [(-0.33) - 0.99]	0.11 ± 0.26 [(-0.95) - 0.80]	0.8532
% Low risk (< 0.11) [f]	45	46	
% Medium risk (0.11 – 0.21)	26	18	
% High risk (> 0.21)	29	36	
TNF-α (pg/mL) [c]	31.1 ± 25.1 (8.9 – 135)	17.1 ± 15.4 (4.2 – 109)	**0.0063**

Maximum and minimum values are shown in parenthesis; [a] 1-OHP value < 0.24 µmol/mol creatinine; [b] 1-OHP value > 0.24 µmol/mol creatinine [c] Geometric mean ± standard deviation; [d] Indicative values of isolated systolic hypertension were observed (PAS > 140 mm Hg, PAD < 90 mm Hg); [e] Arithmetic mean ± standard deviation; [f] Referring to risk of CVD. Student's unpaired t tests were performed to compare between groups.

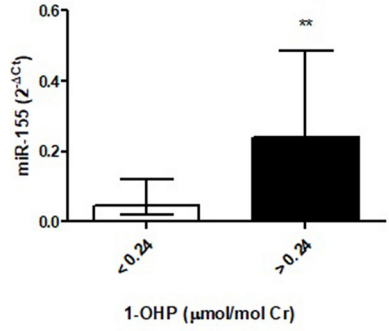


Figure 1: miR-155 expression levels in women grouped according to the reference value of 1-0HP. •• p= 0.0065; Geometric mean with 95% CI is shown (Student's unpaired t test).

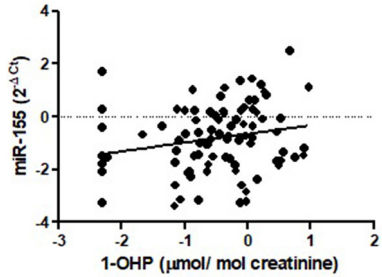


Figure 2: Relationship between 1~HP and plasma miR-155 expression. r= 0.21; p= 0.0432. Data were log transfonned (Pearson correlation test).

0.0246 and p= 0.0065, respectively) (Figure 1; supplementary material). To assess the relation between 1-OHP concentrations and miRNAs expression, Pearson correlation analysis was performed in the entire dataset, resulting in a positive significant association: miR-155 (r= 0.21; p= 0.0432) (Figure 2; supplementary material). Finally, the relation between 1-OHP levels and miR-155 expression levels was assessed adjusting for the clinical variables through a multivariable linear regression analysis performed on the entire dataset. Predictor variables for miR-155 modeling were BMI, glucose, AIP, TNF-α and 1-OHP and the adjusted R-squared was 0.26 (p= 0.0006) (Table 2; supplementary material). These results indicate that 26 % of the variability of miR-155 expression can be explained by predictor variables. Our study has several limitations, starting with its transversal nature which can cause bias in terms of the temporal variation between exposure to PAHs and the levels of the measured variables. In addition, given that the source of PAHs is wood smoke, we cannot rule out the influence of other contaminants present in the mixture. miR-155 is expressed in several cell types, but given that these comprise cells of the immune system, mainly macrophages, as well as vascular cells (endothelial cells and VSMC), it is imperative to establish the real significance of our in a cardiovascular context. To our knowledge, this is the first research showing a relation between miR-155 from immune cells and PAHs exposure via biomass smoke. Despite the limitations of the study, the results add insights and perspectives to the effort of elucidating the mechanisms involved in vascular damage related to exposure to PAHs.

REFERENCES

Jongeneelen, F.J., 2001. Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. Ann. Occup. Hyg. 45, 3–13. https://doi.org/10.1016/S0003-4878(00)00009-0

Li, X., Kong, D., Chen, H., Liu, S., Hu, H., Wu, T., Wang, J., Chen, W., Ning, Y., Li, Y., Lu, Z., 2016. miR-155 acts as an anti-inflammatory factor in atherosclerosis-associated foam cell formation by repressing calcium-regulated heat stable protein 1. Sci. Rep. 6, 21789. https://doi.org/10.1038/srep21789

Pruneda-Alvarez, L.G., Ruíz-Vera, T., Ochoa-Martínez, A.C., Pérez-Maldonado, I.N., 2016. Urinary trans-trans muconic acid (exposure biomarker to benzene) and hippuric acid (exposure biomarker to toluene) concentrations in Mexican women living in high-risk scenarios of air pollution. Arch. Environ. Occup. Health 1–8. https://doi.org/10.1080/19338244.2016.1272539

Sierra, M.I., Valdes, A., Fernandez, A.F., Torrecillas, R., Fraga, M.F., 2016. The effect of exposure to nanoparticles and nanomaterials on the mammalian epigenome. Int. J. Nanomedicine 11, 6297–6306. https://doi.org/10.2147/IJN.S120104

Wei, Y., Nazari-Jahantigh, M., Neth, P., Weber, C., Schober, A., 2013. MicroRNA-126, -145, and -155: A Therapeutic Triad in Atherosclerosis? . Arterioscler. Thromb. Vasc. Biol. 33, 449–454. https://doi.org/10.1161/ATVBAHA.112.300279

Conjugated bilirubin increases T regulatory cell suppressor function during hepatitis A virus infection through a T cell immunoglobulin and mucin domain containing proteins- mediated mechanism

Jorge L Trujillo-Ochoa[1], Karla F Corral-Jara[2,3], Claudia L Charles[3], Arturo Panduro[2,3], Nora A Fierro[2,3]

[1]Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Guadalajara, Mexico

[2]Hospital Civil de Guadalajara, Guadalajara, Mexico

[3]Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico

egipto12_96@hotmail.com; karlaf.jara@gmail.com; karlaf.jara@gmail.com; hclcharles@gmail.com; biomomed@cencar.udg.mx; biomomed@cencar.udg.mx; noraalma@gmail.com; noraalma@gmail.com

Keywords: Bilirubin, Hepatitis A, T regulatory cells, TIM-3, TIM-1

Hepatitis A virus (HAV) infection is a major cause of viral hepatitis worldwide and, the pediatric population is the one with the greater risk of contracting infection. The exact mechanisms that contribute to the resolution of the disease during the acute phase are poorly understood. It is accepted that the final elimination of the virus is mediated by a specific and effective host immune response and, a decreased T regulatory cells (Tregs) frequency is associated with liver injury in acute viral hepatitis. In this context, during liver-tropic viral diseases the host immune response may also be affected by metabolic components. Particularly, during acute viral hepatitis, heme degradation is interrupted leading to the deregulation of bilirubin internalization and excretion by hepatocytes, which results in increased conjugated bilirubin (CB) values. Until 30 years ago, bilirubin was considered to be toxic waste product. However, anti-inflammatory and immuno-modulatory effects have been associated to this metabolite. We recently reported that CB plays a role in adjusting STAT-1 and STAT-5 function and inducing changes in the proportion of Tregs during acute HAV infection. These observations suggest that Tregs function may be related to the resolution of the disease and that CB may be responsible for this effect.

The T cell immunoglobulin and mucin domain containing proteins (TIMs) play a key role in regulating T cell responses. TIM-1 and TIM-3 also recognized as hepatitis virus cellular receptors 1 and 2 respectively, are both expressed on hepatocytes and lymphoid cells. TIM-1 together TCR and co-stimulatory receptors regulates the expansion and effector functions of T helper cells. TIM-3, via its interaction with galectin-9 (GAL-9),

has been proposed to promote immunological tolerance as a result of an enhanced Tregs activity. The involvement of TIM proteins in the mechanisms related to the crosstalk between CB and Tregs during HAV infection has not been elucidated yet.

Herein, in vitro stimulation of Tregs from healthy donors with CB resulted in an increase of Tregs suppressor function. However, treatment with CB in vitro did not induce Tregs de novo in T cells from healthy donors and no changes in IL-10 or TGF-beta secretion were found after treatment. The augment in Tregs function related to CB was in agreement with the finding that, following CB treatment in vitro, TIM-1 and TIM-3 expression in Tregs from healthy donors was augmented. This led us to compare the frequency of CD4+CD25+TIM1+ and CD4+CD25+TIM3+ T cells in peripheral blood lymphoid cells from acute HAV-infected pediatric patients with abnormal CB values, relative to the frequency of the same population in healthy individuals. HAV infection was associated with an increased frequency of CD4+CD25+ T cells expressing TIM-1+ and TIM-3+. Additionally, when Tregs from healthy donors were induced to over-express TIM-3 following CB treatment, an augment in their ability to suppress CD4+ T cell proliferation was found. Interestingly, when Tregs over-expressing TIM-3 as a result of CB incubation were treated with an anti-TIM-3 antibody, which was previously described to have blocking functions in vivo, Tregs were significantly less efficient to suppress CD4+ T cell proliferation. Thus, TIM-3 expression allowed us to identify a subset of Tregs with increased suppressor function following CB-treatment in-vitro. Furthermore, the proportion of CD4+ T cells from HAV-infected patients expressing GAL-9+ increased relative to that in healthy donors, suggesting that TIM-3/GAL-9 interactions may constitute a mechanisms by which CD4+ T cells are suppressed during HAV infection via modulation of Treg activity as a result of CB immunomodulatory and anti-inflammatory functions. This finding was supported by the observation that following CB treatment in vitro, IL-17 and IL-21 secretion by CD4+ T cells from healthy donors showed a trend towards a reduction which in turn, may result of the less degree of CD3-epsilon, Syk, and CREB phosphorylation found in CD4+ T cells from HAV-infected pediatric patients with CB greater than 2 mg/dL.

In conclusion our data strongly suggest that, during HAV infection, CB plays a role in modulating Tregs function in a mechanism related to the expression of TIM-protein family members, specifically TIM-1 and TIM-3 on the cell surface, ultimately leading to an anti-inflammatory effect.

A better understanding of HAV-related immune pathogenesis, particularly those aspects related to metabolic components might provide insights for other infectious diseases affected by host metabolites including hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis E virus (HEV) infections. This may help to decipher why HAV does not persist in the infected host, whereas other liver-tropic viruses do.

The role and expression of long-noncoding RNA in periodontal disease: A pilot study

Javier González-Ramírez[1], Fausto Sanchez-Munoz[2], Gustavo Martínez-Coronilla[1], Ana Gabriela Leija-Montoya[1], Ulises Rieke-Campoy[1], María de Lourdes Montaño-Pérez[1], Ernesto Beltrán-Partida[1], Yolanda Bojorquez-Anaya[1], Nicolás Serafin-Higuera[1], Estefania Guadalupe Jacobo-Garcia[1]

[1]Universidad Autónoma de Baja California, Mexicali, Mexico
[2]Instituto Nacional de Cardiología, Mexico City, Mexico
javier.gonzalez.ramirez@uabc.edu.mx; fausto22@yahoo.com; gustavoj@uabc.edu.mx; gabriela.leija@uabc.edu.mx; rieke@uabc.edu.mx; lourdes.montano@uabc.edu.mx; beltrane@uabc.edu.mx; yolandabojorquez@uabc.edu.mx; nserafin@uabc.edu.mx; jacoboe22@uabc.edu.mx

Keywords: Periodontitis, miRNA, long non-coding RNA, OIP5-AS1, non-coding RNA's

Periodontitis is a chronic inflammatory disease that compromises the integrity of the tissues supporting the teeth, known as periodontium (Pihlstrom, Michalowicz, & Johnson, 2005). Despite all the research on chronic inflammation in periodontitis, the detailed mechanisms of the pathogenesis of chronic inflammation remain unknown (Yucel-Lindberg & Bage, 2013). Among the new expression regulators, the non-coding RNAs have emerged as important determinants in the control of the inflammatory process (Marques-Rocha et al., 2015). Due to the above, we determined the expression profiles of different long non-coding RNAs (lncRNAs) that are known or are predicted to be implicated in inflammation. In this study, we take periodontal samples of healthy subjects, patients with gingivitis and with periodontitis. In both disease groups, the lncRNA OIP5-AS1 expression levels were lower than levels from healthy subjects ($P < 0.05$). This study reveals new insight on the relative levels of OIP5-AS1 lncRNA in the periodontal tissue which has important implications for their potential development as biomarker.

REFERENCES

Marques-Rocha, J. L., Samblas, M., Milagro, F. I., Bressan, J., Martinez, J. A., & Marti, A. (2015). Noncoding RNAs, cytokines, and inflammation-related diseases. FASEB J, 29(9), 3595-3611. doi: 10.1096/fj.14-260323

Pihlstrom, B. L., Michalowicz, B. S., & Johnson, N. W. (2005). Periodontal diseases. Lancet, 366(9499), 1809-1820. doi: 10.1016/S0140-6736(05)67728-8

Yucel-Lindberg, T., & Bage, T. (2013). Inflammatory mediators in the pathogenesis of periodontitis. Expert Rev Mol Med, 15, e7. doi: 10.1017/erm.2013.8

Novel immunoregulatory points to control fasciolosis

Teresa Freire[1], Paula Carasi[1], Juan Ernesto Rodríguez Camejo[1], Sofía Frigerio[1], Valeria Da Costa[1], Veronica Elisa Noya[1], Natalie Brossard[1], Juan J. Garcia-Vallejo[2], Ignacio Anegon[3]

[1]Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[2]VU University Medical Center, Amsterdam, Netherlands
[3]INSERM UMR1064 Centre de Recherche en Transplantation et Immunologie, Nantes, France
tfreire@fmed.edu.uy; paulacarasi@gmail.com; erodriguez@fmed.edu.uy; frigeriosofia@gmail.com; valedacosta21@gmail.com; veronicanoyaro@gmail.com; brossardnatalie@gmail.com; jj.garciavallejo@vumc.nl; ignacio.Anegon@univ-nantes.fr

Keywords: Fasciola hepatica, IL-10, immuneregulation, Heme-oxygenase, C-type lectin receptor heme-oxygenase

Fasciola hepatica, also known as the liver fluke, is a trematode that infects livestock and humans causing fasciolosis, a zoonotic disease of increasing importance due to its worldwide distribution and high economic losses. This parasite immunoregulates the host immune system by inducing a strong Th2 and regulatory T immune response by immunomodulating dendritic cell (DC) maturation and alternative activation of macrophages. We have identified some of the molecular mechanisms that contribute to the parasite immunoregulation, and that could constitute control points to prevent fasciolosis. One of this is the C-type lectin receptor macrophage Gal/GalNAc lectin-2 (MGL2) expressed on DCs and macrophages during the infection. This receptor recognizes the Tn antigen (GalNAc-Ser/Thr) on parasite components and mediates different process, such as parasite-derived molecules uptake and signaling, conferring MGL2-positive cells the ability to express regulatory cytokines (IL-10, TNFα and TGFβ) and a variety of regulatory markers. Moreover, MGL2+CD11c+cells expand parasite-specific IL-10-producing CD4 T cells and suppress Th1 polarization, and contribute to parasite survival. The second point of control that we have identified is the heme-oxygenase-1 (HO-1), the rate-limiting enzyme in the catabolism of free heme, which is upregulated in liver and peritoneum during F. hepatica infection and regulates the host inflammatory response. HO-1 is expressed by two different antigen presenting cells in the peritoneum of infected animals: alternatively activated macrophages and regulatory DCs. Furthermore, the pharmacological induction of HO-1 with the synthetic metalloporphyrin CoPP promoted F. hepaticainfection increasing the clinical signs associated with the disease. In contrast, treatment with the HO-1 inhibitor SnPP protected mice from parasite infection, indicating that HO-1 plays an essential role during F. hepaticainfection. Finally, HO-1 expression during F. hepaticainfection

was associated with TGFβ and IL-10 levels in liver and peritoneum, suggesting that HO-1 controls the expression of these immunoregulatory cytokines during infection favoring parasite survival in the host. These results contribute to the elucidation of the immunoregulatory mechanisms induced by F. hepatica in the host and provide alternative checkpoints to control fasciolosis.

PP2A B55ß limits the lifespan of self-reactive and pathogen-specific CD8 T cells through the pro-apoptotic molecule HRK

Noe Rodriguez-Rodriguez[1,2], J. Alejandro Cisneros-Segura[1,3], Sokratis A. Apostolidis[4], Marcela Esquivel-Velázquez[1], Tran Nguyen[4], George C Tsokos[4], Jorge Alcocer-Varela[1], Florencia Rosetti[1], José Carlos Crispín[1]

[1]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[3]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[4]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States
noahrodriguez2@gmail.com; alejandro.jcs25@gmail.com; socrates.apostolidis@gmail.com; esquivel.marcela@gmail.com; tnguyennb@gmail.com; gtsokos@bidmc.harvard.edu; jorgealcocer2@gmail.com; florencia.rosettis@incmnsz.mx; carlos.crispina@incmnsz.mx

Keywords: Apoptosis, T cells, PP2A, PPP2R2B, HRK

The magnitude and length of immune responses are closely controlled to avoid unnecessary inflammation and tissue damage. Apoptosis, in particular of activated T and B cells, represents an essential mechanism in place to curb the immune response. Several cell-intrinsic and environmental factors have been proposed to regulate the initiation of apoptosis in activated T cells. In particular, abundance of cytokines and growth factors, as well as antigen availability. However, how T cells integrate these cues, or rather their absence, and opt for programmed cell death is poorly understood.

Previous work from our lab has identified B55ß, a regulatory subunit of the serine/threonine phosphatase PP2A, as a regulator of cytokine withdrawal-induced cell death in human activated T cells. The aim of this work was to investigate whether B55ß controls the survival of T lymphocytes during immune responses and to dissect the molecular mechanisms through which it induces apoptosis.

To determine whether B55ß regulates the survival of T cells during acute immune responses, we generated OT-I mice with conditional deficiency of Ppp2r2b2, the gene that encodes B55ß. Wild-type (WT) and B55ß-deficient OT-I cells were isolated and adoptively transferred to CD45.1 recipient mice who were infected with ovalbumin-expressing Listeria monocytogenes (LM-OVA). There were no differences in the expansion or activation of OT-I cells at Day 7 post-infection (p.i.). However, at Days

14 and 30 p.i., absence of B55ß caused a significant accumulation of activated IFN-gamma-producing OT-I cells. This phenomenon was associated to decreased apoptosis of B55ß-deficient T cells (ex vivo and in vitro).

Signaling pathway analyses demonstrated that cytokine withdrawal causes the inactivation of the kinase AKT and that this regulatory modification, exerted by dephosphorylation, is dependent on the presence of B55ß. Complementary experiments in cells that lacked B55ß or that over-expressed it showed that B55ß upregulation during cytokine withdrawal leads to AKT modulation and transcriptional activation of the FoxO1/3a factors. Interestingly, the expression of only one pro-apoptotic gene, Hrk, was shown to depend on B55ß in human and murine T cells. Moreover, knockdown experiments demonstrated that Hrk is required for activated T cells to undergo apoptosis induced by cytokine deprivation.

Finally, we interrogated whether B55ß was able to control the survival and activation of self-reactive CD8 T cells by transferring WT or B55ß-deficient OT-I effector cells into mice that express OVA under the control of the rat insulin promoter. Lack of B55ß granted OT-I cells an increased survival capacity that was associated with aggressive pancreatic infiltration and development of diabetes.

Our results indicate that PP2A B55ß represents the molecular link that allows activated T cells to undergo apoptosis at the end of acute immune responses, when cytokine concentrations dwindle. Failure to upregulate B55ß causes an abnormal accumulation of activated T cells with enhanced pro-inflammatory potential. Moreover, we have dissected the pathway through which B55ß regulates cellular survival and discovered a novel non-redundant role of the BH3-only protein Hrk.

Hypermethylation of PPP2R2B represents a novel mechanism by which chronic inflammation perpetuates itself

Iris K. Madera-Salcedo[1], Beatriz Sánchez-Hernández[1], Yevgeniya Svyryd[1], Marcela Esquivel-Velázquez[1], Noe Rodriguez-Rodriguez[1,2], Emmanuel Dávalos-Estela[1], Rogelio Cuervo-Campos[1], Osvaldo M. Mutchinick[1], Jorge Alcocer-Varela[1], Florencia Rosetti[1], José Carlos Crispín[1]

[1]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
maderasalcedo@gmail.com; beugeniash@yahoo.com.mx; ysvyryd@yahoo.com.mx; esquivel.marcela@gmail.com; noahrodriguez2@gmail.com; iversix@gmail.com; rogelio_cuervo@hotmail.com; osvaldo@unam.mx; jorgealcocer2@gmail.com; florencia.rosettis@incmnsz.mx; carlos.crispina@incmnsz.mx

Keywords: Apoptosis, Autoimmunity, T cells, CpG methylation, PPP2R2B

The B55ß regulatory subunit of the phosphatase PP2A, encoded by PPP2R2B, controls apoptosis in activated T cells during cytokine withdrawal. Accordingly, mice with a T cell conditional deficiency of Ppp2r2b accumulate activated T cells at the end of acute immune responses. In humans, B55ß is required for apoptosis in response to cytokine withdrawal, and in patients with the autoimmune disease systemic lupus erythematosus (SLE), failed expression of B55ß is associated with T cell resistance to apoptosis.

The aim of this work was to evaluate the kinetics of expression of B55ß in T cells from patients with systemic autoimmune diseases (i.e. SLE, rheumatoid arthritis [RA], and Sjögren's syndrome [SS]) and analyze the genetic and epigenetic mechanisms that regulate its transcription in health and disease.

We included 86 patients (SLE=34; RA=28; SS=24) and 25 healthy controls matched by age and gender. Patients fulfilled classification criteria (SLE 1997 ACR; RA: 1987 ACR; SS: American-European consensus 2002) and all individuals signed informed consent forms. Peripheral blood-isolated T cells were activated (OKT3 + anti-CD28) and expanded in the presence of IL-2 during 10 days. At that point, DNA and RNA were isolated and apoptosis was estimated according to Annexin V binding and membrane permeability to Sytox orange. Cells were then deprived of IL-2 and analyzed at 24 and 48 hours.

IL-2 deprivation induced the expression of B55ß in T cells from healthy controls, but failed to do so in ~50% of patients with SLE and RA. B55ß transcription in response to IL-2 withdrawal was normal in patients with SS. As previously reported, T cells from SLE patients exhibited resistance to apoptosis. Moreover, apoptosis induced by IL-2 deprivation was significantly impaired in patients with RA and SLE that failed to upregulate B55ß. This phenomenon was more marked in CD8+ and CD4-CD8- (double negative) T cells. These results indicate that B55ß induction is abnormal in patients with SLE and RA and that such defect is associated with resistance to apoptosis of activated T cells.

We analyzed the PPP2R2B locus, in order to identify regulatory elements that could be linked to the failed transcription of the gene in patients with autoimmunity. First, we focused on a CAG repeat sequence whose expansion has been linked to increased PPP2R2B expression. To this end, we quantified the number of repeats present in each allele of PPP2R2B from patients (RA and SLE) and controls. The frequency of repeats was normal in all individuals studied. Next, we analyzed a large CpG island located in a conserved sequence that includes the first exon and the flanking intronic regions. Using methylation-sensitive PCR, we detected a higher degree of DNA methylation in patients with SLE. In order to confirm these findings, and to identify the specific regulatory elements, we performed pyrosequencing. We identified a region, comprised by 4 CpGs, located between -50 to -70 bps from the transcription start site of the gene, that was highly methylated in patients whose T cells failed to transcribe B55ß, but showed no methylation in cells that upregulated B55ß in a normal manner.

Our results suggested that factors extrinsic to the T cell, for example inflammatory mediators, could affect its DNA methylation at the level of PPP2R2B. To test this, we isolated T cells from healthy donors and cultured them in the presence of different cytokines. Compared to T cells expanded in the presence of IL-2, the addition of pro-inflammatory cytokines caused a decrease in the rate of apoptosis and a reciprocal increase in the methylation of PPP2R2B.

Collectively, our results have identified a gene whose regulation affects the sensitivity of activated T cells to undergo apoptosis during cytokine withdrawal. Moreover, our data has revealed an epigenetic mechanism through which T cells activated in inflammatory milieus acquire resistance to apoptosis. This may enable them to exert more intense pro-inflammatory activities, but in the context of autoimmunity, it may represent the pathogenic basis of a positive feedback loop that perpetuates inflammation.

CD38 enhances the activation and proliferation of regulatory T cells

Jocelyn Carolina Pérez Lara[1], Gabriela Lopez-Herrera[2], Enrique Espinosa[3], Hector Romero[4], Juan Carlos Rodriguez Alba[1]

[1]Universidad Veracruzana, Xalapa, Mexico
[2]Instituto Nacional de Pediatria, Mexico City, Mexico
[3]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
[4]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
JOCELYN1908@HOTMAIL.COM; lohegabyqbp@gmail.com; hector.enrique.espinosa@gmail.com; hromero@cinvestav.mx; jcra19@yahoo.com

Keywords: Autoimmunity, Immunotherapy, IL-10, Treg cells, CD38

CD38 is a 42-kilodalton (kDa) transmembrane glycoprotein widely expressed in hematopoietic tissue, it possesses receptor and ecto-enzyme activities. The stimulation of CD38 induces mobilization of intracellular calcium, activation, proliferation, differentiation or apoptosis of several cell types, suggesting an important role in immunological cells development. Engagement of CD3+ T cells with agonistic anti-CD38 mAb induces its proliferation and IL-2 and IL-10 production. CD38 is involved in numerous physiological and pathological process, including autoimmune diseases. Regarding, some reports, have suggested that the absence CD38 accelerates the onset of autoimmunity. Nevertheless, the mechanisms associated to CD38 in order to regulate or control the autoimmune process are still unknown. Some evidence reveals that CD38 is highly expressed by subsets with regulatory properties such as Regulatory T (Treg) cells, in addition, data from our group shows that Regulatory B (Breg) cells does express CD38, suggesting that these subpopulations requires CD38 in order to perform its functional role. In this work, we hypothesized that CD38 is required for the Treg development and the CD38 engagement induces activation and proliferation of Treg cells using the B6.CD38-/- mice and the wild-type strain as control. Our results shown that CD38 expression did not depend on levels of CD25 but rather on the FoxP3 expression. In addition, Treg CD38+ cells (60%) showed higher CD69 and CTLA-4 expression compared to the Treg CD38- cells, indicating, that the phenotypic features of CD38+ Treg cells are consistent with an effector Treg phenotype. Finally, our data showed that combined use of anti-CD38 (NIMR-5) plus anti-CD3/CD28 and IL-2 enhance the proliferation of Treg cells and IL-10 production. In resume, our results suggested that CD38 expression is required in the function of FoxP3+Treg and consequently could help in the autoimmune disorders control.

The dietary flavonoids: Hesperidin and naringenin, decreases the activation and proliferation of splenocytes b and t cells from a lupus prone-mice

Amayrani Alia Abrego[1], Hector Romero[2], Enrique Espinosa[3], Juan Carlos Rodríguez Alba[1]

[1]Universidad Veracruzana, Xalapa, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
[3]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
amayrani.abrego@gmail.com; hromero@cinvestav.mx; hector.enrique.espinosa@gmail.com; jcra19@yahoo.com; jcra19@yahoo.com

Keywords: Hesperidin, proliferation, activation, Naringenin, Dietary flavonoids, Lupus-prone mice

The flavonoids Hesperidin and Naringenin are widely distributed in the citrus fruits. Several reports suggest that both compounds possess a wide range of biological properties, include immunomodulation effects. Some evidence shows that other flavonoids as Astilbin reduces the activated B and T cells percentages, diminish the production of autoantibodies and proinflammatory cytokines in "in vivo" administration into autoimmune disease mice models. Additionally, it has also been suggested that Naringenin increases the suppressive activity of regulatory T cells. Yet, it is unclear how they could regulate immune system functions, these studies suggest that flavonoids may participate in the control of autoimmune diseases and could be used as a therapy with natural compounds. The aim of this work was to evaluate the ability of Naringenin and Hesperidin to modulate the proliferation, activation and cytokines production of B and T lymphocytes from lupus-prone mice model (B6.MRL.Fas lpr/lpr) compared to the wild type control, performing "in vitro" assays after the LPS, PMA/Ionomycin, anti-IgM F(ab)2 or anti-CD3/CD28 stimuli. Our results show that splenocytes from B6.MRL.Fas/lpr mice treated with flavonoids and stimulated 72 hrs across their TLR4 reduces expression of molecules such as CD80 and CD86 in B cells, but does not shown any effect in their proliferation. Besides, these flavonoids decreased the percentage of T cells with activated phenotype CD44highCD62Llow from the autoimmune-prone mice, whereas, in the wild type has not shown similar effect. We next investigated whether flavonoids have an proliferative effect upon BCR or TCR stimulation. We found that both flavonoids produced a significant reduction in the T and B cell after activation through their BCR or TCR. Finally, the results show a decreased in the amounts of pro-inflammatory cytokines. Collectively, these results suggest that the flavonoids have a great potential to be used in the treatment of autoimmune diseases.

Generation of a mouse with lymphoproliferative disorder plus B cell deficiency (B6.μMT/lpr) as a model for the study of autoimmunity and immunodeficiency

Michelle Stephany Rodriguez Hernández[1], Carlos Arturo Gallardo Hernandez[1], Hector Romero[2], Juan Carlos Rodríguez Alba[1]

[1]*Universidad Veracruzana, Xalapa, Mexico*

[2]*Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico*

Factorywnk@gmail.com; carlos_26_arturo@hotmail.com; hromero@cinvestav.mx; jcra19@yahoo.com

Keywords: Autoimmunity, Lymphoproliferative Disorders, mouse models, MRL/Lpr mouse, B cells absence, μMT mouse

Autoimmune-prone murine models mimic the pathology of diverse human diseases such as Systemic Lupus Erythematosus (SLE) and are helpful in the study of cellular and genetic mechanisms involved in these disorders. In this way, several lupus-like models have been created and one of the most studied is the B6.MRL.Faslpr/lpr, this one is characterized by autoantibodies production and autoimmune symptoms mediated by B cells. In addition, the role of other populations as T lymphocytes, dendritic cells, monocytes or eosinophils is still studied in order to understand the processes that leads or enhances this disease. In this work, we decided to generate a transgenic mouse keeping the lymphoproliferative disorder (B6.MRL.Faslpr/lpr), adding a mutation in the heavy chain of the IgM B-cell-receptor that affects the B lymphocytes development, in order to create a mouse that allow us to study both autoimmune and immunodeficiency diseases, focusing in the T cell development. Our strategy was to cross the B6.MRL. Faslpr/lpr strain with the B6.Ighmtm1Cgn strain (also known as μMT), following the Jackson Laboratory protocol. Firstly, the homozygote mice for mutant gene A (in this case B6.MRL.Faslpr/lpr) was mate with the homozygote for mutant gene B (B6. Ighmtm1Cgn) the resulting progeny was 100% heterozygous for both genes. Next, the double heterozygous were mate together to obtain several genotypes, then we PCR genomic DNA of each mice, the probability to get our final genotype (B6.MRL.Faslpr/lpr.μMT) was 12.5% for each gene (A and B) as long as the total number of progeny be 16. Finally, the heterozygous for gen A was cross with their homologous, likewise for the gen B (about 25%) until we got the B6.MRL.Faslpr/lpr.μMT mice.

Inhibins regulate peripheral induction of regulatory T cells through modulation of dendritic cells

Marisol De La Fuente-Granada[1], Sandra Ortega[1], Roxana Olguín-Alor[1], Juliana Idoyaga[2], Laura Bonifaz[3], Eduardo García-Zepeda[1], Soldevila Gloria[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]School of Medicine, Stanford University, Stanford, United States
[3]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
mdelafuente@iibiomedicas.unam.mx; amyrisortega@gmail.com; rolguinalor@gmail.com; jidoyaga@stanford.edu; labonifaz@yahoo.com; garciaze@ervidor.unam.mx; soldevi@unam.mx

Keywords: Dendritic Cells, Peripheral Tolerance, peripheral, Tregs, inhibin

Inhibins and Activins, members of the Transforming Growth Factor superfamily, participate in the immune system as key regulators of several cellular functions. We have recently reported that Inhibins play an important role in dendritic cell (DC) maturation and function. Inhα-/- DCs showed an impaired upregulation of MHCII and co-stimulatory molecules after LPS treatment, diminished in vitro and in vivo migration, reduced capacity to activate T CD4+ cells in vitro and lower DTH responses in vivo. These results suggested that Inhibins may play an important role maintaining the balance between inflammation and tolerance, through DCs, such as regulatory T cell (Treg) induction. Analysis ex vivo of CD4+ T subpopulations in Inhα-/- mice, showed an increase of Tregs (CD4+CD25+) in lamina propria (colon, mesenteric lymph nodes (MLN), and peripheral lymph nodes (LN) under homeostatic conditions. Interestingly, the percentage of iTregs (CD4+CD25+Foxp3+Helios-) in lamina propria (colon) and mesenteric lymph nodes (MLN) was significantly increased. To understand if these findings were related to differences in DCs, ex vivo analysis of DCs was performed. Inhα-/- mice showed an increase number of CD103+ DCs in MLN, which are known to favor the induction of iTregs. Furthermore, in vitro differentiated DCs (BMDCs) and ex vivo obtained CD11c+ DCs from Inhα-/- mice, stimulated with LPS, and co-cultured with naïve T cells in the presence of anti-CD3 and TGFb induced a higher generation of iTregs (3 times more) in vitro compared with their wild type counterparts. To analyze the relevance of this findings in vivo, we transferred CD4+OT-II+CD45.1+ naïve T cells into Inh±-/- CD45.2+ mice, followed by an immunization, with ovalbumin (OVA) or antigen targeting DC (anti-DEC205-OVA), and in both cases, groups with and without adjuvant (Cholera Toxin (CT)) were included. In these experiments we found an increase in the number of OT-II+CD45.1+ Treg induced

in the Inh±-/- recipient mice compared to Inh±+/+ in the groups immunized with anti-DEC205-OVA, anti-DEC205-OVA + CT and OVA, showing that Inhibin deficient DCs are prone to induce a tolerogenic response in vivo. These results point out the importance of Inhibins in the control of peripheral tolerance.

Regulation of tolerogenic markers on human dendritic cells by MYC

Paulina A. García-González[1,2], Katina Schinnerling[1,2], Jaxaira Maggi[1,2], Ahmed Murtaza Mehdi[3], Hendrik Johannes Nel[3], Diego Francisco Catalán[1,2], Ranjeny Thomas[3], Ricardo A Verdugo[1], Juan C Aguillon[1,2]

[1]Universidad de Chile, Santiago, Chile
[2]Millennium Institute on Immunology and Immunotherapy (MIII), Santiago, Chile
[3]Diamantina Institute, University of Queensland, Brisbane, Australia
garcia.gonzalez.pa@gmail.com; katina.schinnerling@gmail.com; jaxaira.maggi@gmail.com; a.mehdi@imb.uq.edu.au; h.nel@uq.edu.au; dfcatalan@med.uchile.cl; ranjeny.thomas@uq.edu.au; raverdugo@u.uchile.cl; jaguillo@med.uchile.cl

Keywords: Immune Tolerance, Metabolism, Transcription Factors, Transcriptome, Tolerogenic dendritic cells

Introduction: Tolerogenic dendritic cells (tolDCs) have become a promising therapeutic tool for immune regulation in the context of autoimmune diseases. The cellular mechanisms by which these tolerogenic DCs (tolDCs) exert their regulatory function are well known, however the molecular mechanisms driving the differentiation and function of these cells are still poorly understood. Additionally, many essential cellular processes seem to be regulated by metabolic processes and though we know there is a metabolic switch between immature and mature DCs, little is known about the metabolic responses that prevail in tolerogenic dendritic cells. Our aim was to identify molecular regulators and metabolic pathways associated with a tolerogenic function in monocyte-derived DCs.

Methods: Transcriptional profiling of Dex-modulated/MPLA-activated monocyte-derived dendritic cells (DM-DCs) was performed, and Differentially Expressed (DE) genes were identified compared to unstimulated/immature DCs. Functional enrichment and pathway overrepresentation was performed using Ingenuity Pathway Analysis. Expression of genes of interest was confirmed through qPCR, and protein levels as well as intracellular concentration of zinc and reactive oxygen species (ROS) were determined by flow cytometry. Transactivation of target genes of in DCs was blocked for 48 hours after which expression of tolerogenic markers upregulated in DM-DCs was assessed. Unstimulated DCs and MPLA-mature DCs were used as controls in all the experiments.
Results: DM-DCs exhibited characteristic tolerogenic features and a particular transcriptomic profile, from which we identified several genes related to tolDCs differentiation and regulatory function that were upregulated. IPA upstream regulators analysis

revealed MYC as a potential transcriptional regulator of our dataset, which was also amongst most the upregulated DE genes in DM-DCs. We confirmed MYC differential expression on DM-DCs at a transcriptional as well as at a protein level, and its blockade lead to the downregulation of tolerance-related markers such as JAG1 and IDO1. On the other hand, functional enrichment analyses showed that fatty acid oxidation, oxidative metabolism and zinc homeostasis were the main biological functions represented in the data set. Supporting this, we also found higher intracellular expression of ROS and Zinc on DM-DCs compared to mature (M-DCs) and unstimulated controls (DCs).

Conclusion: DM-DCs tolerogenic profile appear as result of a coordinated modulation of tolerance and inflammation related-genes, transcriptional regulators such as MYC, and metabolic processes, all of which ultimately drive the regulatory features of these cells.

Relationship between the expression of HSP60 and the Unfolded protein response in THP-1 monocytes

José Pablo Romero-López, María Lilia Dominguez-López, Luis Antonio Jiménez-Zamudio, Ethel García-Latorre

Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico
pablorolo30@gmail.com; ldmguez@yahoo.com.mx; lajimenezz@gmail.com; ethelagarcia@hotmail.com

Keywords: Monocytes, Unfolded Protein Response, er stress, protein misfolding, Heat shock protein 60

The unfolded protein response (UPR) is a cellular mechanism that tries to protect the cell against acute endoplasmic reticulum (ER) stress in conditions such as protein misfolding. On the other hand, "Spondyloarthritis" (SpA) is an autoinflammatory disease with a strong association with the HLA-B27 molecule. This molecule tends to misfold during its synthesis in the ER, this phenomenon results in ER stress and activation of the UPR with further IL-23 production (1).

Our group have found that plasma and synovial fluid samples from patients with SpA have high titers of antibodies against the heat shock protein of 60 kDa (HSP60) of enterobacteria like Klebsiella pneumoniae and Yersinia enterocolitica, likewise, we did a bioinformatic analysis of some residues of the protein, then, we found that the residues 389–397, 360–368 and 282–290 could be candidates of B cell recognition and that the antibodies of the patients did not recognize the protein lacking the 282–290 residue. Interestingly, this residue (282–290) that was potentially recognized by the antibodies of the patients and the 360–368 one, have an arginine in the position two of their sequence, which is crucial for every peptide to be presented in the B27 pocket, so apart of being recognized by antibodies, this peptide can indeed be presented to T cells (2,3).

Remarkably, antibodies against human HSP60 have also been reported in patients with SpA (4), so, considering that HSP60 is an important intracellular chaperone, and that it is possible that human and bacterial HSP60 share important epitopes, we aimed to determine if the expression of HSP60 is associated with the activation of the UPR in a cell line of human monocytes.

First, the homology between the human HSP60 and the HSP60 from Klebsiella was determined by BLAST, resulting in a 50% of identity of the complete protein. The homology for the peptide 282–290 resulted in 67% of identity and of 75% for the

389–397 one, so it is possible that the human HSP60 can indeed be related with immune activation in these patients.

ER stress was induced in three million of THP-1 cells with 1, 3 and 5 μg/mL of tunicamycin for six hours, one group of cells was also activated with 100ng/mL of LPS from E. coli, then, the expression of HSP60, BiP and CHOP was determined by western blot. Also, the expression and location of HSP60 was analyzed by confocal immunofluorescence.

The results showed an increase of the expression of HSP60 dependent of the concentration of tunicamycin added to the culture, this expression of HSP60 was associated with the increase of the expression of CHOP, suggesting a role of the PERK/ATF4 pathway. Furthermore, the activation of the stressed monocytes with LPS induced an important induction of HSP60.

These results could help to understand how the ER stress and the UPR can be involved in the modulation of the immune response mediated by heat shock proteins.

REFERENCES

1. Colbert, R. A., Tran, T. M., & Layh-Schmitt, G. (2014). HLA-B27 misfolding and ankylosing spondylitis. Molecular Immunology, 57(1), 44–51. https://doi.org/10.1016/j.molimm.2013.07.013
2. Cancino-Diaz, M. E., Pérez-Salazar, J., Domínguez-López, M. L., Escobar-Gutiérrez, A., Granados-Arreola, J., Burgos-Vargas, R., Jiménez-Zamudio L, García-Latorre, E. (1998). Antibody response to Klebsiella pneumoniae 60 kDa protein in familial and sporadic ankylosing spondylitis: role of HLA-B27 and characterization as a GroEL-like protein. Journal of Rheumatology, 25(9), 1756–64.
3. Cancino-Diaz, M., Ayala-Narvaez, H., Burgos-Vargas, R., Reyes-López, A., Castillo-Tovar, L., Domínguez-López, M. L., Granados-Arreola J., Jiménez-Zamudio L., García-Latorre, E. (2000). Recognition of B cell epitopes of the Klebsiella pneumoniae GroEL-like protein by HLA-B27 positive subjects. Microbial Pathogenesis, 28(4), 211–220.
4. Hjelholt, A., Carlsen, T., Deleuran, B., Jurik, A. G., Schiottz-Christensen, B., Christiansen, G., & Birkelund, S. (2013). Increased levels of IgG antibodies against human HSP60 in patients with spondyloarthritis. PLoS ONE, 8(2), e56210. https://doi.org/doi: 10.1371/journal.pone.0056210

TIM3 expression in polarized M1/M2 human macrophages

Aquiles Ranferi Ocaña Guzmán, Lucero Ramón-Luing, Alonso Torres Hatem, Fausi Bulhusen Rodriguez, Isabel Sada Ovalle

Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
arocana@iner.gob.mx; ramonluing@yahoo.com.mx; hatem.alonso@hotmail.com; fausi.bul@gmail.com; isadamx@gmail.com

Keywords: Immune Tolerance, Macrophage Activation, TIM-3, M1 and M2 macrophages, galectin 9

Macrophages are phagocytic cells, which are localized through the whole human body. Monocytes give rise to these terminally differentiated cells. Macrophages have several functions to maintain immune homeostasis such as, host protection, tissue repair, phagocytosis, clearance and secretion of diverse soluble factors, which contribute to innate and adaptive defenses against infection and counteract inflammatory processes, while distinct secreted signals, restore tissue homeostasis and promote subsequent repair.1,2 To perform protective functions and repair damaged tissues, monocytes and macrophages express a wide range of surface, vacuolar and cytosolic receptors for recognition and uptake of host-derived (damage signals) and foreign particles; many of these receptors also facilitate phagocytosis, endocytosis, sense viral, bacterial and parasitic molecules.3 Activation through theses receptors, results in signals to control or regulate functions in the same macrophage and distant targets cells. Their phagocytic capacity is variable, and may even be undetectable, but provides a well-developed machinery to internalize, degrade and store cargo such as poorly degraded foreign particles 4.

In addition, macrophages expressing regulatory surface molecules can attenuate or inhibit cell activation, which is considered a tolerance mechanism critical to preserve tissue homeostasis.

The TIM-3 protein was initially known as a membrane-specific marker for Th1 and Tc1 lymphocytes, 5 but its expression was soon identified in other cell lines. Today, we know that TIM-3 is expressed in monocytes, macrophages, dendritic cells, NK cells, and even diverse cells in different tumor types. 6–10 In macrophages, it has been shown that the TIM-3/Gal-9 interaction can negatively regulate TLR-mediated signaling reducing the IL-12 production, increasing IL-23, and reducing phosphorylation of STAT-1, while also augmenting activation of STAT-3. 11

Macrophages undergo specific differentiation depending on the local tissue environment. The M1 macrophage phenotype is characterized by the production of pro-inflammatory cytokines, high production of reactive nitrogen and oxygen intermediates, and promotion of Th1 responses. In contrast, M2 macrophages are characterized by their involvement in parasite control, tissue remodeling, immune regulation, tumor promotion and efficient phagocytic activity. Some authors suggest that M1 macrophages could re-direct to the M2 phenotype, since they lose or reduce many of the protective functions in cancer, thus promoting tumor development and progression 12. These events are enabled by the characteristic plasticity of macrophages that – unlike lymphocytes which are polarized toward one of the known effector profile and suffer significant modifications at the level of the chromatin – show a genetic profile that changes depending on the type, concentration, and time of exposure to diverse stimuli 5,6

Hypothesis: The Tim-3 expression is different between M1 vs M2 polarized macrophages.

Methods: To evaluate if the Tim-3 expression between M1 (INF-g 20 ng/mL + LPS 50 ng/mL) vs M2 (IL-4 20 ng/mL + IL-13 20 ng/mL) macrophages was different, we tested cells from 6 healthy donors, and perform an ELISA assays (IL-1b, IL-10) to probe functional profile of polarized macrophages. Then, Tim-3 and Gal9 expression were measured by flow cytometry each 24 hours, for 3 days. Additionally, mRNA expression of Tim-3, Gal-9. Finally, as we know that Tim-3 and Gal-9 can be found in soluble form, we perform ELISA assay to evaluate possible differences in the concentration of this proteins in supernatants of polarized macrophages.

Results: There were no differences in the frequency and mean fluorescence intensity (MFI) of Tim-3 and Gal9 positive cells at 0, 24 and 48 hours, although the Tim-3 MFI at 72 hours was decreased in M1 polarized macrophages (mean=9181relative fluorescence) this difference was no statically significant. In contrast, M2 polarized macrophages showed a similar Tim-3 MFI (mean=11661 RF) as the unstimulated control (mean=15770 RF) at 72 hours.

When we evaluate the TIM3 expression at mRNA level, we didn't found differences between M1 vs M2 macrophages at 0, 24, 48 or 72 hours. In contrast, the Gal-9 relative expression, increased at 24 hours after INFg/LPS stimuli (med=2.426; max=3.463, min=2) compared with IL-4/IL-13 treatment at 24 hours (med=1.141; max=1.325, min=0.837).

Finally, we perform an ELISA assay to evaluate the secreted amount of Tim-3. Our results showed an important decrease in sTim-3 in the supernatant of M1 macrophages (mean=1681 pg/mL) compared with M2 (6094 pg/mL) and unstimulated macrophages (3956 pg/mL) at 72 hours.

Conclusion: Tim-3 expression was not different at the membrane level, but we observed a lower percentage of TIM3 positive cells in M1 macrophages at 72 hours compared with M2 and unstimulated macrophages. A similar result was obtained when soluble levels of Tim-3 were measured. M1 macrophages secreted less TIM3 than M2 and unstimulated macrophages. In accordance with previous reports, our results can suggest that TIM3 must be reduced to obtain a complete activation process in a proinflammatory microenvironment. The changes showed in membrane-bound and soluble TIM3 can suggest that TIM3 protein expression, is reduced when a proinflammatory stimulus is present. Finally, Gal-9 mRNA showed a higher expression in M1 macrophages at 24 hours after INFg/LPS stimuli. Previously has been showed that Gal-9 expression can be induced by INFg. However, the increase in relative expression not correlates with membrane-bound or soluble form of Gal-9, which can suggest the existence of a post-transductional regulation mechanism in Gal-9 expression.

Our results suggest that TIM3 expression and subsequent release to the extracellular medium can be a regulatory mechanism. Has been demonstrated that Tim-3 expression and signaling, can impact in macrophages and lymphocytes activation. In this work, we realize that soluble form of TIM3, deceased when M1 polarization occurs, which is in accordance with the inhibitory role of TIM3. Now we can evaluate if TIM3 soluble protein, can affect the activation of immune cells.

PD-1 regulates the expression of HELIOS and EGR2 in self-reactive CD8+ and CD4$^-$CD8$^-$ T cells

Noe Rodriguez-Rodriguez[1,2], Sokratis A. Apostolidis[3], Edgar Montes-Servín[4], Óscar Arrieta[4], George C Tsokos[3], Florencia Rosetti[2], José Carlos Crispín[2]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[3]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States
[4]Instituto Nacional de Cancerología (INCan), Tlalpan, Mexico
noahrodriguez2@gmail.com, socrates.apostolidis@gmail.com, ragde789@hotmail.com, oscararrietaincan@gmail.com, gtsokos@bidmc.harvard.edu, florencia.rosettis@incmnsz.mx, carlos.crispina@incmnsz.mx

Keywords: Peripheral Tolerance, CD8 T cell, PD-1, Helios, Egr-2

Self-reactive CD8 T cells that encounter cognate antigen in peripheral tissues become inactivated, downregulate CD8, and express high levels of PD-1 (Pdcd1), becoming PD-1+ double negative (DN, CD4$^-$CD8$^-$ TCR-αβ+) T cells. An analogous subset of PD-1+ DN T cells induced by self-antigens is scarcely found in healthy mice, but increased in autoimmunity. Because PD-1 is pivotal for immune tolerance and self-reactive DN T cells express high levels of this immune checkpoint molecule, we investigated its role in the generation of DN T cells induced by self-antigen and in the regulation of their expansion and pro-inflammatory capacities.

Genetic deletion of Pdcd1 led to a significant increase in the fraction of Ki-67+ and IFN-gamma+ cells, specifically within the DN T cell population. Analogously, transfer of PD-1-deficient OT-I CD8+ T cells into mice that constitutively express ovalbumin as a self-antigen, resulted in an increased expansion of DN T cells with an elevated production of pro-inflammatory cytokines (i.e. IL-2, TNF-alpha, and IFN-gamma). Together, these results indicate that PD-1 curbs the proliferation and cytokine production of self-reactive DN T cells during steady state and following cognate antigen encounter.

Further analyses revealed that HELIOS (Ikzf2) and EGR-2, two transcription factors associated with immune tolerance, were specifically expressed by self-reactive DN T cells upon self-antigen encounter. Interestingly, absence of PD-1 or its receptor PD-L1 (Cd274), as well as blockade of PD-1 during early antigen encounter, abrogated the upregulation of HELIOS and EGR-2, suggesting that signaling via PD-1 is necessary

for their induction in an in vivo system. Complementary in vitro experiments confirmed that the presence of PD-L1 during T cell activation enabled a stable induction of HELIOS and EGR2. Additional in vitro assays demonstrated that PI3K/AKT inhibition, exerted by PD-1 signaling, promotes the expression of HELIOS and EGR-2 in an SHP-1- and SHP-2-dependent manner.

To determine the relevance of our findings, we analyzed T cells from patients with the autoimmune disease systemic lupus erythematosus (SLE). Compared to healthy controls, patients with SLE had an abnormally high abundance of PD-1+ DN T cells within peripheral blood T lymphocytes. Moreover, HELIOS expression was deficient and, accordingly, expression of the proliferation marker Ki-67 was increased in DN T cells from patients with SLE. Finally, we interrogated the effects of PD-1 blockade in patients with non-small cell lung carcinoma, by analyzing DN T cells in their blood before and after treatment with pembrolizumab. In them, anti-PD-1 administration caused a significant reduction in the frequency of HELIOS+ DN T cells together with a reciprocal elevation of Ki-67+ DN T cells.

In conclusion, signaling through PD-1 is pivotal to preserve tolerance by curbing the pro-inflammatory activity of self-reactive CD8+ and DN T cells. By promoting the expression of HELIOS and EGR-2, PD-1 restrains the expansion of DN T cells, hindering their pathogenic potential. Defects in PD-1 expression and function, through the mechanism described here, may contribute to the pathogenesis of autoimmune diseases and represent an interesting target for immune modulation.

Role of regulatory T cells in the inflammatory process associated to breast cancer in obesity

Aleida Núñez Ruiz, Flor Trinidad Sánchez Brena, Cynthia Paola López Pacheco, Naray Alejandra Acevedo Domínguez, Eduardo García Zepeda, Gloria Soldevila Melgarejo

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
aleidanuru@gmail.com; flowereft@gmail.com; cyntlop@hotmail.com; nara_2305@hotmail.com; garciazeg6@icloud.com; soldevi@servidor.unam.mx

Keywords: Exosomes, Obesity, breast cancer, regulatory T cells, adiposomes

In 2012 WHO declared that breast cancer is the disease with the highest incidence and mortality in female population in the world. It is known that one of the risk factors of this disease is obesity. The one of the mechanisms proposed to explain predisposition, is that hypertrophy suffered by adipocytes induces a hypoxic microenvironment which, added to the secretion of pro-inflammatory cytokines, promotes tumor growth and increases the metastatic ability of cancer cells [1]. The tumor microenvironment is constituted by different cellular subpopulations such as fibroblasts, adipocytes, endothelial cells and immune cells; Among them, regulatory T cells play an important role in cancer, as they may inhibit the anti-tumoral response and promote tumor establishment and growth. It has been demonstrated that breast cancer patients have an increased percentage of regulatory T cells in peripheral blood and in the tumor infiltrate, and this correlates with poor prognosis of patients [2]. Another mechanism by which breast cancer cell can interact with the microenvironment is the release of exosomes, extracellular vesicles of endosomal origin with 30–120 nm of diameter that play a role in apoptosis, stem cell stimulation, cancer cell invasion and metastasis, immune cell regulation and drug resistance [3]. Recently, a study has shown that cancer-associated adipose tissue in breast cancer can release exosomes and that co-cultures with breast cancer cell line induces CREB phosphorylation and a stronger propensity to form mammospheres [4]. However, there is no information about the mechanisms by which adipocytes (adiposomes) could affect regulatory T cells and the tumoral microenvironment. Here, we evaluated the influence of adipocyte exosomes in Treg generation/expansion recruitment and function during the development of breast cancer. We used a murine model of diet-induced obesity or mice fed with normal diet (60% and 10% fat) in tumor growth by inoculation of a breast cancer cell line (E0771; ER+) and analyzed the percentage and number of regulatory T cells (CD4+CD25highFOXP3+) in peripheral organs (spleen, lymph nodes and sentinel lymph node), tumor and adipose tissue; also, we characterized exosomes from adipocytes by western blot (WB), transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA).

Our results show a significative increase in weight and a diminished percentage of peripheral blood regulatory T cells in mice fed with obesogenic diet compared with mice fed with control diet, in accordance with previous reports [5,6]. Regarding tumor growth, obese mice presented tumors with increased size and weight compared to lean mice. On the other hand, subpopulation analysis in the breast cancer mouse model, showed a significant decrease in the percentage of CD4+ and CD8+ populations in peripheral lymph nodes in obese mice, while regulatory T cells CD4+CD25highFoxP3+ were increased in the sentinel lymph node of obese mice in comparison with lean mice which reflects the influence of the tumor microenvironment, as this lymph node is near the tumor. Concerning the infiltrating cells within the adipose tissue, a diminished percentage of CD3+, CD4+, and CD4+CD25highFoxp3+ was observed in obese mice, in agreement with the model of obesity previously reported [6]. Analysis of cells infiltrating the tumor showed a significant decrease of CD4+, CD8+ and CD4+CD25+ cells in obese mice compared to lean mice, suggesting that obesity may impair the anti-tumoral response. In contrast, the percentage of regulatory T cells were increased in the obese mice, favoring the tumor escape from the immune system. Our group proposes that adipocytes, by releasing adiposomes may act on Tregs, regulating their number and/or function and thereby influencing the tumor microenvironment. To address this issue, adiposomes were obtained from the supernatant of primary adipocytes and OP9-differentiated cells showing a diameter between 40–120nm and expression of CD9 and CD81, that are markers characteristic of exosomes. In summary, our data demonstrate that obesity affects tumor development and peripheral and intratumoral lymphocyte subpopulations, specifically Tregs, which could be directly related to the regulation of the tumor microenvironment and anti-tumoral responses. We also demonstrate that adipocytes are able to release exosomes that can be purified for functional analysis. The effect of exosomes from adipocytes on regulatory T cells and their role in the development of breast cancer is currently under investigation.

REFERENCES

1. Park, J., et al., Obesity and cancer--mechanisms underlying tumour progression and recurrence. Nat Rev Endocrinol, 2014. 10(8): p. 455-65.
2. Ehara, M., et al., Regulatory T cells and breast cancer : Implications for immunopathogenesis. Cancer Metastasis Rev, 2010; 29:569-579
3. Kucharzewska, P., et al., Emerging roles of extracellular vesicles in the adaptive response of tumour cells to microenvironmental stress. J Extracell Vesicles 2013;2:20304
4. Jeurissen, S., et al., The isolation of morphologically intact and biologically active extracellular vesicles from the secretome of cancer-associated adipose tissue. Cell Adhesion and migration, 2017. 11(2): p. 196-204.
5. Feuerer, M., et al., Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med, 2009. 15(8): p. 930-939.
6. Deiuliis, J., et al., Visceral Adipose Inflammation in Obesity Is Associated with Critical Alterations in Tregulatory Cell Numbers. Plos One, 2011. 6(1): e16376

Suppression of Immune Regulation in a Murine Model of Progressive Pulmonary Tuberculosis

Vasti Lozano Ordaz[1,2], Rogelio Hernandez Pando[2], Brenda Marquina-Castillo[2], Jorge Alberto Barrios-Payan[2], Dulce Adriana Mata[2]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
loov1288@hotmail.com; rhdezpando@hotmail.com; brenda.marquina@gmail.com; qcjbp77@yahoo.com.mx; dulmat@yahoo.com.mx

Keywords: Tuberculosis, mouse model, Treg cells, HO-1, IDO (indoleamine 2,3-dioxygenase)

(Mycobacterium tuberculosis) is the etiologic agent of tuberculosis (TB). Mtb infects one third of the world´s population and it's estimated that annually causes the death of 1.3 million persons. Many biological and immunological aspects of TB are not completely elucidated, such as the complex process of immunoregulation mediated by the Treg cells (regulatory T cells), the enzymes IDO (indoleamine 2,3-dioxygenase) and HO-1 (hemoxygenase 1). When BALB/c mice are infected with a high dose of Mtb strain H37Rv by the intratracheal route, a rapid progressive disease is developed. In this model of TB we previously studied the kinetics of Treg, IDO and HO-1 along the infection. In the lungs of tuberculous animals during the progressive phase there was high percentage of Treg cells, however a high gene expression of FoxP3 and high expression and production of HO-1 and IDO particularly in macrophages. In this study Treg cells was deleted with anti-CD25 mAb (PC61 clone), and the activity of IDO and HO-1 was inhibited using the specific inhibitors, 1-MT (1-methyl-D,L-tryptophan) to block IDO and ZnPp (zinc protoporphyrin-IX) to suppress HO-1. In comparison with control animals, mice treated with anti-CD25 or 1-MT showed a significant decrease of bacilli loads. Moreover, increased proinflammatory Th1 cytokines and decreased Th2 cytokines was observed in mice with depletion of Treg, and in which the enzyme IDO and HO-1 was inhibited; no differences in the percentage of pneumonic area observed in either case. We conclude that the regulatory response is involved in the control of inflammation, as previously described. The new finding of this work is that regulatory response promotes proliferation of mycobacteria in the lung.

Hepatic expression of miR-21, HIF1A and TGF beta ligands (TGFB1, TGFB2, TGFB3) and receptors (TBRI, TBRII, BAMBI) in broilers with cold induced pulmonary hypertension

Javier González-Ramírez[1], Fausto Sánchez-Muñoz[2], Gabriel Ruiz-Castañeda[3], Norma Dominguez- Avila[4], Ana Gabriela Leija-Montoya[5], Nora Fernandez-Jaramillo[6], Yaneli Juárez-Vicuña[2], Ricardo Marquez-Velasco[2], Rafael Bojalil[2], Román Espinosa-Cervantes[3], Fausto Sánchez[3]

[1]Universidad Autónoma de Baja California, Mexicali, Mexico
[2]Instituto Nacional de Cardiología, Mexico City, Mexico
[3]Universidad Autónoma Metropolitana, Mexico City, Mexico
[4]Universidad Autónoma Agraria Antonio Narro, Saltillo, Mexico
[5]Universidad Autónoma de Baja California
[6]Universidad Autónoma de Baja California Benemérita Universidad Autónoma de Puebla, Puebla, Mexico
javier.gonzalez.ramirez@uabc.edu.mx; fausto22@yahoo.com; zoogenia@yahoo.com.mx; neda2007@gmail.com; gabriela.leija@uabc.edu.mx; nora.fdz@hotmail.com; yaneli2608@hotmail.com; marquezric@hotmail.com; rafaelbojalil@gmail.com; espinosa@correo.xoc.uam.mx; sygf2122@correo.xoc.uam.mx

Keywords: pulmonary hypertension, TGF-beta, miR-21, BAMBI, HIF1A

Background: In broilers, as a consequence of pulmonary arterial hypertension, it may be observed cardiac failure followed by congestive hepatopathy and ascites. In addition, incidence of pulmonary arterial hypertension may be increased by cold ambient temperature.

Material and Methods: In liver tissue from six weeks of age broilers submitted to 1 week of low ambient temperature (14oC), we determined by RT-qPCR the gene expression of ligands (TGFB1, TGFB2, TGFB3) and receptors (TBRI, TBRII, BAMBI) of the transforming growth factor beta and the hypoxia-inducible factor 1 alfa (HIF1A) genes, and of the microRNA-21. The mRNA relative quantification of target genes was conducted using ACTB and HPRT as reference genes and the $2-\Delta\Delta Ct$ method. Data were tested by two-way ANOVA with interaction effects (treatment: Control vs cold ambient temperature; Cardio-hepatic condition: Healthy birds, birds with cardiac failure, and birds with cardiac failure and ascites).

Results: HIF1A was affected by the treatment X Cardio-hepatic condition interaction ($P<0.05$). HIF1A levels were higher only in ascitic birds submitted to cold ambient

temperature while no differences were observed between healthy, cardiopath and ascitic birds raised at control room temperature ($P>0.05$). The treatment effect was significant only on miR-21, with higher expression levels in cold temperature raised birds as compared to Controls ($P<0.05$). Cardio-hepatic condition effect was significant on TGFB2 and TGFB3 ($P<0.05$), expression level for both genes was higher in ascitic than in healthy birds. The treatment, the cardio-hepatic condition and the interaction effects were not significant on TGFB1, TBRI, TBRII and BAMBI expression genes. Most partial correlation coefficients among TGF-beta family genes were positive ($P< 0.05$). Finally, significant partial correlations between miR-21, TGFB1, TBRI, TBRII and BAMBI were found ($P<0.05$).

Conclusions**:** TGFB2 and TGFB3 increased expression are implied in the progression from heart failure to ascites, whereas miR-21 was related to cold exposure response. Further work is needed to elucidate the positive correlation of miR-21 with TGFB1, TBRI, TBRII and BAMBI, but not with TGFB2 and TGFB3.

Modulation of acute and chronic inflammation by the mineralocorticoid receptor in animal models

Natalia Munoz Durango[1], Alexis M Kalergis[1], Claudia Riedel[2], Evelyn Liliana Jara Fernández[1], Luis Fernando Venegas Salas[2], Geraldyne Alessandra Salazar[1]

[1]Pontificia Universidad Católica de Chile, Santiago, Chile
[2]Universidad Andrés Bello, Santiago, Chile
natydurango@gmail.com; akalergis@bio.puc.cl; criedel99@yahoo.com; jaraevelyn@gmail.com; luis.venegas.s@gmail.com; geraldyne.salazar@gmail.com

Keywords: Aldosterone, sepsis models, EAE model, immune regulation and homeostasis, Mineralocorticoid Receptor signaling

The mineralocorticoid receptor (MR) is a ligand dependent transcription factor. MR has been conventionally related with the control of water and electrolyte homeostasis to keep blood pressure through aldosterone activation. However, MR is also expressed in cells of the immune system, where it responds to stimulation or antagonism, controlling immune cell function. We previously showed that dendritic cells (DCs) express and respond to MR stimulation with aldosterone by secreting proinflammatory cytokines that controls Th17 polarization. Currently, we are evaluating whether aldosterone induces a pro-inflammatory state in DCs by promoting overexpression of Toll like receptors (TLR). We found that aldosterone treated DCs express higher levels tlr4 than MRKO DCs or DCs treated with MR antagonists. Also, these cells express higher levels of CD86 and secreted higher amounts of IL-6 than controls. Finally, in sub-lethal model of endotoxic shock, we found that pre-treated mice die earlier after LPS challenge in contrast to vehicle, mainly due to a multi-organic failure. Additionally, in a model of chronic inflammation, such as EAE, we observed that myeloid MR conditional knock-out mice (MyMRKO) mice developed higher clinical EAE scores compared to control. MyMRKO showed a higher percentage of CD45+ leukocytes infiltrating the central nervous system (CNS), principally CD45+CD8+. In vitro co-cultures showed higher levels of IL17A and IFNγ secretion from CD8+cells stimulated with MyMRKO DCs than control. In conclusion, our data suggest that aldosterone and its receptor play an important role controlling the function of immune cells in acute and chronic diseases.

Role of adiposomes in the inflammatory process associated breast cancer in obesity

Flor Trinidad Sánchez-Brena, Aleida Núñez Ruiz, Cynthia Paola López-Pacheco, Eduardo García Zepeda, Gloria Soldevila

Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
flowereft@gmail.com; aleidanuru@hotmail.com; cyntlop@hotmail.com; garciaze@unam.mx; soldevi@unam.mx

Keywords: Adipose Tissue, Inflammation, breast cancer, exosome, TAMs

Introduction: In Mexico, breast cancer is the most common malignancy in women with an incidence of 28.7% and the first cause of mortality (14.8%). An important risk factor for its development is obesity, with a prevalence in Mexico of 71.3%. In adipose tissue, the profile of adipokines, cytokines and chemokines depends on the stage of tissue inflammation; thus, in lean adipose tissue, adipocytes produce secretion of anti-inflammatory adipokines such as adiponectin, favoring infiltration by M2 macrophages and Th2 lymphocytes, which secrete anti-inflammatory cytokines such as IL-10, IL-1Ra, IL-4, and IL- 13. On the contrary, in an obese adipose tissue it is observed that the adipocytes suffer hypertrophy and hyperplasia derived from the excessive accumulation of triglycerides, which leads to a secretion of pro-inflammatory adipokines such as leptin and chemokine CCL2. In addition, some of the adipocytes undergo necrosis and are phagocytosed by macrophages, favoring their polarization towards an M1 profile (TNF-α, IL-6, IL-1β and IL-12), causing the infiltrated cells of the immune system to differentiate towards a Th1 phenotype. There is extensive evidence demonstrating the effect of tumor-derived exosomes on the immune cells. Exosomes are extracellular vesicles of endosomal origin with 30–120 nm of diameter, highly enriched in micro RNAs, that have been shown to play a role in tumor progression. Recently, it has been described that similarly to other cell types, adipocytes can release exosomes (adiposomes), which may have an effect on the phenotype and function of surrounding cells, including immune cells. Given the association of obesity with the development of breast cancer, we propose that adipocytes can influence the immune cells recruited to the tumor through the production of adiposomes within the tumor microenvironment, and therefore may favor an anti-inflammatory milieu that promotes breast cancer progression.

Hypothesis: In obesity, adipocyte-derived exosomes (adiposomes) regulate the breast cancer tumor microenvironment by affecting the polarization of tumor-associated macrophages M1/M2.

Objetive: To evaluate the effect of adiposomes in the polarization of macrophages in vitro and in vivo and their role in modulating the tumor microenvironment in breast cancer.

Materials and Methods: In vitro: Adipocytes were differentiated from the mouse mesenchymal cell line OP9. The adiposomes were isolated by ultracentrifugation, purified by size exclusion chromatography and characterized by Transmission Electron Microscopy (TEM), Nanoparticle Tracking Analysis (NTA) and western blot. The polarization of macrophages M1/M2 is performed from in vitro bone marrow derived macrophages in the presence or absence of adiposomes and under basal or inflammatory conditions (proinflammatory cytokines/palmitic acid) and analyzed by flow cytometry. In vivo: mouse model of obesity and breast cancer was used where C57BL/6 mice are fed 60% fat diet for 10 weeks and then mice are implanted with cell line E0771 ER+. The M1/M2 macrophages in the tumor are evaluated by immunohistochemistry and the M1/M2 macrophages in lymph nodes, spleen, adipose tissue and tumor by flow cytometry.

Results and Conclusion: Exosomes of adipocytes differentiated in vitro (OP9 derived) have a size of 40–120 nm in diameter and an approximate concentration of 4x10e9 particles/ml. In contrast, the adiposomes obtained from mouse adipose tissue showed a larger size 50–250 nm in diameter and their concentration is 4.5x10e10 particles/ml and 8.9x10e10 particles/ml of mice with normal diet and fat diet, respectively. In addition, these exosomes expressed CD9, CD81 and TSG101, which are characteristic markers of exosomes.

In relation to the in vivo model, at week 10 with fat diet, the mice show a significant increase in weight (relative increase of 30%) and percentage of monocytes F/480+ CD11b+ in peripheral blood compared to mice with normal diet. In a 14-hour fast the mice have blood glucose levels with a mean of 113 mg / dL, which indicates that they have not developed hyperglycemia. Interestingly, the tumor of obese mice is 50% over weight and size compared to lean mice.

On the other hand, analysis of F/480+ CD11b+ macrophages in lymph nodes and spleen of the breast cancer model shows an increase in the percentage of these cells, reflecting the systemic influence of the tumor. The analysis of the cellular infiltrate both in the tumor and in the adipose tissue reveals an increase of macrophages F480+ CD11b+ in the obese mouse in comparison with the lean mouse, which could be favoring the tumor growth.

We propose that adipocytes, by releasing a large amount of adiposomes, have an effect on macrophages, regulating their number and/or function and, thus, influencing the tumor microenvironment. In summary, our data show that obesity can affect tumor development and subpopulations of cells such as macrophages in different tissues, which could be directly related to the regulation of the tumor microenvironment. We also show that adipocytes can release exosomes that can be purified for functional analysis. The effect of adiposomes on macrophages and their role in the development of breast cancer is currently under investigation.

CD5-CK2 signaling axis is involved in the generation of regulatory T cells

Erica Paulina Burgueño-Bucio[1], Roxana Olguín-Alor[1], Samuel Mendoza-Martínez[1], Carlos Alberto Mier-Aguilar[1,2], Chander Raman[3], Eduardo Alberto García-Zepeda[1], Gloria Soldevila[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
[3]University of Alabama at Birmingham, Birmingham, United States
qfb.burgueno@gmail.com; rolguinalor@iibiomedicas.unam.mx; mmartsam@hotmail.com; cama.mier@gmail.com; chanderraman@uabmc.edu; garciaze@servidor.unam.mx; soldevi@unam.mx

Keywords: Foxp3, regulatory T cells, CD5, CK2, Knockin mice

Regulatory T cells (Tregs) control autoimmune responses and exacerbated or unwanted inflammatory responses. These cells can be generated in the thymus (tTregs) or from naïve T cells in the periphery (pTregs). Tregs are characterized by the constitutive expression of CD25 and Foxp3 transcription factor. Interestingly, among all T cells subpopulations, Tregs present the highest levels of CD5 and Casein Kinase 2 (CK2) expression of.

CK2 is a constitutive active and ubiquitous serine/threonine kinase. It has been reported that conditional deletion of CK2 in Tregs leads to an impairment in their suppressive function towards Th2 responses [1]. Also, pharmacological inhibition or genetic silencing of CK2 in CD4 T cells results in impaired Th17 and enhanced Treg differentiation, resulting from a reduction in STAT3 activation and the PI3K/Akt/mTOR pathway [2–4]. CK2 is constitutively associated to CD5 at its carboxy-terminal region (S458-S461) and phosphorylates CD5 constitutively and after activation [5, 6].

CD5 is a 67kDa glycoprotein expressed in all mature and immature T lymphocytes and B1a cells. Regarding Tregs, the absence of CD5 (CD5KO) results in an increased tTreg generation and an increased protection from apoptosis of this population, probably due to a reduced phosphorylation of Akt [7]. CD5KO Tregs seems to present a greater suppressive activity, as demonstrated in a model of DSS-induced colitis showing a lower clinical score, which correlated with increased Foxp3 expression in colonic Tregs [8]. Henderson and colleagues reported that CD5lo or CD5KO CD4+ CD25- T cells present a reduced ability to differentiate towards Tregs. However, the domains within CD5 cytoplasmic region involved in promoting iTreg generation are currently unknown. It is well-known the regulatory axis between Th17/Treg differentiation . Recent data

has shown that CD5-CK2 signaling promotes Th17 differentiation, however it remains to be investigate whether this pathway regulates iTreg induction.

In this work we sought to determine if CD5-CK2 signaling axis has an effect in the generation of tTregs and pTregs. For this, we analyzed cells from thymus, lymph nodes (LN), mesenteric lymph nodes (mLN) and spleen for the expression of Foxp3 that identifies Tregs and the expression of Helios to identify tTregs (Helios+) from pTregs (Helios-). Consistent with what was previously reported [9], and in contrast to CD5KO, there were no differences in the percentage and total numbers of thymic Tregs between CD5ΔCK2 and CD5WT mice in any lymphoid organs analyzed. Interestingly, we found that both tTregs and pTregs in CD5ΔCK2 mice showed reduced CD5 expression, as CD5 expression is regulated by TCR signaling strength [10], the lower CD5 expression in CD5ΔCK2 Tregs would suggest these cells received a weaker signal through TCR. We also found that spleen and LN Tregs from CD5ΔCK2 mice present a reduced Foxp3 expression both in tTregs and iTregs. As Foxp3 expression has been correlated with suppressive function and as CK2 plays a role in Treg suppressive activity, these data would suggest that CD5ΔCK2 Treg may be impaired in their function.

CD5 has also been implied in Treg induction, however which of CD5 functional domains is responsible for these effect is unknown. It has been reported that the inhibition of CK2 in CD4+ T cells leads to an increased Treg generation. To address if CD5-CK2 signaling has an effect in Treg generation, CD4+ CD25- T cells from spleen and LN where purified and stimulated with α-CD3 and α-CD28 in the presence of TGFβ in vitro. Treg generation was enhanced in the absence of CD5-CK2 signaling and generated Tregs presented an increased Foxp3 expression,these is in accordance with published data using pharmacological inhibition or genetic ablation of CK2. Moreover, CD5ΔCK2 Tregs express reduced levels of CD5, like the ex vivo analysis showed. Interestingly, CD5ΔCK2 Tregs hypo-proliferated, consistent with what was been observed in CD4 T cells in this same mouse [11]. We are currently analyzing the mechanism by which this effect is achieved, by measuring the phosphorylation of multiple signaling molecules, including Akt and different STATs.

Altogether these data suggest that CK2 recruitment to the cytoplasmic tail of CD5 is relevant for iTreg induction. The fact that the absence of CD5-CK2 signaling does not reproduce the effects observed in CD5KO mice indicates that other signaling domains of CD5 may play a role in nTreg and iTreg generation.

REFERENCES

1. Ulges, A., et al., Protein kinase CK2 enables regulatory T cells to suppress excessive TH2 responses in vivo. Nat Immunol, 2015. 16(3): p. 267-75.

2. Ulges, A., et al., Protein kinase CK2 governs the molecular decision between encephalitogenic TH17 cell and Treg cell development. Proc Natl Acad Sci U S A, 2016. 113(36): p. 10145-50.
3. Jang, S.W., et al., Casein kinase 2 is a critical determinant of the balance of Th17 and Treg cell differentiation. Exp Mol Med, 2017. 49(9): p. e375.
4. Gibson, S.A., et al., Protein Kinase CK2 Controls the Fate between Th17 Cell and Regulatory T Cell Differentiation. J Immunol, 2017. 198(11): p. 4244-4254.
5. Calvo, J., et al., Human CD5 signaling and constitutive phosphorylation of C-terminal serine residues by casein kinase II. J Immunol, 1998. 161(11): p. 6022-9.
6. Raman, C. and R.P. Kimberly, Differential CD5-dependent regulation of CD5-associated CK2 activity in mature and immature T cells: implication on TCR/CD3-mediated activation. J Immunol, 1998. 161(11): p. 5817-20.
7. Ordonez-Rueda, D., et al., Increased numbers of thymic and peripheral CD4+ CD25+Foxp3+ cells in the absence of CD5 signaling. Eur J Immunol, 2009. 39(8): p. 2233-47.
8. Dasu, T., et al., CD5 plays an inhibitory role in the suppressive function of murine CD4(+) CD25(+) T(reg) cells. Immunol Lett, 2008. 119(1-2): p. 103-13.
9. Mier-Aguilar, C.A., et al., CD5-CK2 Signaling Modulates Erk Activation and Thymocyte Survival. PLoS One, 2016. 11(12): p. e0168155.
10. Azzam, H.S., et al., CD5 expression is developmentally regulated by T cell receptor (TCR) signals and TCR avidity. J Exp Med, 1998. 188(12): p. 2301-11.
11. Sestero, C.M., et al., CD5-Dependent CK2 Activation Pathway Regulates Threshold for T Cell Anergy. The Journal of Immunology, 2012. 189(6): p. 2918-2930.

Exposure to an enriched environment attenuates mouse experimental colitis

Tomás Villaseñor[1], Porfirio Nava[2], Carolina Ivonne Serrano García[2], Leonor Pérez-Martínez[1], Gustavo Pedraza-Alva[1]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
tomas@ibt.unam.mx; pnava@fisio.cinvestav.mx; ivonne40_45@hotmail.com; leonor@ibt.unam.mx; gustavo@ibt.unam.mx

Keywords: Colitis, Inflammation, BDNF, enriched environment, somatosensorial stimulation

It is known that somatosensorial stimulation through exposure to enriched environments enhances central nervous system functions and improves homeostasis (Nithianantharajah & Hannan, 2006; Reichmann, Painsipp, & Holzer, 2013). In addition to the central nervous system, the functions of the immune system are also modulated by exposure to an enriched environment, improving phagocytosis, chemotaxis and attenuating the inflammatory response induced by lipopolysaccharides (Arranz et al., 2010). In this work, we show that exposure to an enriched environment attenuates inflammation in the colon. After dextran sodium sulfate (DSS) or Trinitrobenzenesulfonic acid (TNBS) treatment, animals exposed to an enriched environment showed reduced weight loss, reduced colon shortening and disease activity score than animals housed in a normal environment. Accordingly, the colon of animals exposed to an enriched environment showed reduced epithelial damage, less immune cellular infiltrate, reduced myeloperoxidase activity and secreted lower TNF and IL-1β levels than animals housed in a normal environment. In contrast, colon explants from mice exposed to an enriched environment produce higher levels of the anti-inflammatory cytokine IL-10, in response to DSS or TNBS, than the colon explants from animals housed in standard conditions. In agreement with the fact that exposure to an enriched environment attenuates inflammation and reduces epithelial damage in the colon, we found that animals exposed to the enrich environment had low levels of LPS and other pathogen associated molecular patterns in circulation, compared with animals housed in normal conditions. Together our results show that brain stimulation by exposure to an enriched environment attenuates inflammation in the gut mucosa, resulting in improved epithelial barrier functions.

REFERENCES

Arranz, L., De Castro, N. M., Baeza, I., Maté, I., Viveros, M. P., & De la Fuente, M. (2010). Environmental enrichment improves age-related immune system impairment: long-term exposure since adulthood increases life span in mice. Rejuvenation Research, 13(4), 415–428. https://doi.org/10.1089/rej.2009.0989

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nature Reviews Neuroscience, 7(9), 697–709. https://doi.org/10.1038/nrn1970

Reichmann, F., Painsipp, E., & Holzer, P. (2013). Environmental Enrichment and Gut Inflammation Modify Stress-Induced c-Fos Expression in the Mouse Corticolimbic System. PLoS ONE, 8(1). https://doi.org/10.1371/journal.pone.0054811

Antigen excreted/secreted from Taenia crassiceps modulate the development experimental colitis

Yadira Ledesma-Soto, Blanca Estela Callejas Piña, Sandy Reyes Martínez, Ilse Chávez Soto, Cuauhtémoc Del Ángel Sánchez Barrera, Marissa Calderon Torres, Guillermo Alvarado Hernandez, Luis Ignacio Terrazas

Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico

lesy_790413@yahoo.com.mx; aletse_bianca@hotmail.com; sandy_reyes25@comunidad.unam.mx; ilsebios01@gmail.com; angel.casb@hotmail.com; cmarissacalderon@gmail.com; galvaradohernandez@gmail.com; literrazas@unam.mx

Keywords: Carbohydrates, Colitis, alternatively activated macrophage (M2), Antigen of Taenia crassiceps, Complex E-Cadherina b-Catenina

Colitis is characterized by chronic inflammation of the colonic mucosa. Interestingly, helminth antigens have been shown to represent an important source of molecules that deflect the immune response towards anti-inflammatory profile. Recently has been reported that antigens excreted/secreted from T. crassiceps (TcE/S) may regulate the activation and maturation of dendritic cells, such that might modulate proinflammatory response and therefore the development of experimental colitis.

The aim of this study was evaluated if the glycoprotein present in the antigens excreted/secreted of Taenia crassiceps modulated the development of colitis. Was induced colitis in BALB/c mice through oral administration of dextran sulphate sodium (DSS) 4%. Antigens (TcE/S), antigens depleted of carbohidrates (TcE/Ss/c), antigens depleted of protein (TcE/Ss/p) were administered intraperitoneally, mice with PBS were used as control. The mice were weighed daily for 10 days, TNF-α, IL-10 levels were determined in serum, peritoneal cells and colon were obtained to determine alternatively macrophages activated (AAM) also, the colon was removed to measure the length, On the other hand, tissue damage is assessed by histology. In addition the monocyte infiltrated in the tissue was evaluated by flow cytometry as well as the generation of reactive oxygen species (ROS) and the phosphorylation of NF-kB and P38, we evaluated the integrity of the epithelial barrier by expression of E-Cadherin and β-Catenin.

We found that mice with DSS reduced weight quickly and colon structure is lost, with an infiltration of neutrophils in lamina propria. Additionally, the levels of TNF-α increased, as well as the production of ROS and the phosphorylation of NF-kB and P38. Whereas that, the mice DSS+TcE/S remained constant weight, colon preserved the

structure increased the production of IL-10, the expression the complex E-Cadherina, β-Catenina and reduced the level of TNF-α, phosphorylation NFkB and P38 and reduce the infiltration of neutrophils. Also, generated a population of AAM but when the TcE/S are depleted of carbohidrates and proteins this protection is lost with reduced the expression the AAM.

In **conclusion** we identified that glycoproteins in the TcE/S, decrease the inflammatory processes of experimental colitis due to the generation of AAM, protects the barrier epithelial increasing E-Cadherina and β-Catenina, decreases the production of inflammatory cytokines such as TNF alpha by decrease phosphorylation of NF-κB. Therefore, these findings show that antigens may be important candidates to modulate the development of colitis that may result in the development of colon cancer.

Role of STAT1, STAT6 and IL-4Rα in the development of ulcerative colitis

Ilse Chavez Soto, Yadira Ledesma Soto, Sandy Reyes Martinez, Blanca Estela Callejas Piña, Cuauhtémoc Del Ángel Sánchez Barrera, Yolanda Gonzalez Hernandez, Luis Ignacio Terrazas Valdes

Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla de Baz, Mexico
ilsebios01@gmail.com; lesy_790413@yahoo.com.mx; sandy_reyes25@comunidad.unam.mx; aletse_bianca@hotmail.com; Angel.CASB@hotmail.com; yolandagonzalezhdz@gmail.com; literrazas@campus.iztacala.unam.mx

Keywords: STAT1, inflammatory bowel disease, ulcerative colitis, STAT6, experimental colitis, Dextran Sodium Sulfate, IL-4Rα, transducers and transcription activators (STAT)

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) associated with the secretion of pro-inflammatory cytokines such as TNF-μ, IL-1², IL-17A, IFN-³ but also with the production of characteristic cytokines of a Th2 profile such as IL-13 and IL-4. In these responses the transducers and transcription activators (STAT) also have been implicated in the pathogenesis of colitis. STAT1 and STAT6 are two of the most important pathways that participate in the development of inflammatory or anti-inflammatory responses, respectively; however the role that these molecules play in the UC disease is not yet clear. Thus, the aim of this study was to evaluate the absence of STAT1, STAT6 and IL-4R± in the development of experimental colitis induced with dextran sodium sulfate (DSS). BALB/c (wild type, WT), STAT1-/-, IL-4R±-/- and STAT6-/- mice were used to induce colitis, by oral administration of DSS (4%).The disease activity index (DAI) and the percentage of weight loss was measured daily. At day eight the mice were euthanized and the colon was dissected and divided into different sections: for histology to evaluate the architecture of the colon and immunohistochemistry for the complex E-cadherin/²-catenin. A portion of the colon was cultured overnight and cytokines were measured in the supernatants. Another portion of the colon was used to determinate the expression of MUC2 gene (mucins) by RT-PCR and finally, another part of the colon was used to obtain cells from the lamina propria, which were marked for neutrophils (CD11b+Ly6ClowLy6G+), pro-inflammatory monocytes (CD11b+Ly6ChiLy6G-) and anti-inflammatory monocytes (CD11b+Ly6ClowLy6G-).

We found that WT and STAT1-/- mice displayed the highest severity in the development of DSS-induced colitis, with higher signs of DAI, weight loss and a significant reduction in the length of the colon. At histological level these mice showed a greater cellular infiltrate, composed mainly by neutrophils (CD11b+Ly6ClowLy6G+) and

monocytes (CD11b+Ly6CHiLy6G-). Also we observed a decrease in the percentage of goblet cells, lower expression of the MUC2 gene, and a decrease in the expression of the molecular adhesion complex E-cadherin / β-catenin in the colon epithelium.The least affected groups were IL-4Rα-/- and STAT6-/-. Showing that the absence of the STAT6 molecule favors the protection of the epithelial barrier by promoting greater expression of the molecular adhesion complex E-cadherin/β-catenin, preserving the goblet cells, which express more the MUC2 gene that function as protection for the integrity of the epithelium, reducing the inflammatory process and recruitment of immune cells such as neutrophils and monocytes that contribute to colon damage. However, the absence of STAT1 does not seem to affect the development of colitis. Our data suggest that the IL-4Ra/STAT6 signaling pathway may be a therapeutic target for the treatment of ulcerative colitis.

Mucosal exposure to cigarette components induces intestinal inflammation, colitis and Paneth cells alterations in mice

Loni Berkowitz[1,2], Francisco Javier Salazar[2], Catalina Pardo Roa[2], Geraldyne Alessandra Salazar[2], Gigliola Ramìrez[1], Manuel Alvarez-Lobos[1], Susan M Bueno[2]

[1]Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

[2]Millennium Institute on Immunology and Immunotherapy (MIII), Santiago, Chile

loniberko@gmail.com; salazar.echegarai@gmail.com; cata.pardo.roa@gmail.com; geraldyne.salazar@gmail.com; gigliolaramirez@hotmail.com; manalvarezl@gmail.com; sbueno@bio.puc.cl

Keywords: Intestinal Mucosa, Paneth Cells, Crohn's disease, mouse model, cigarette smoking

Cigarette smoking is the main environmental risk factor associated with the development and progression of Crohn's disease (CD). Although the mechanism is still unknown, some studies have shown that cigarette exposure affects the intestinal barrier of the small bowel, increasing intestinal bacterial translocation. Among the factors that may be involved in this process are Paneth cells (PCs). These cells are specialized epithelial cells of the small intestine that secrete antimicrobial peptides essential for controlling the growth of microorganisms. Alterations in its function are associated with inflammatory processes, such as CD. Considering all of this, we proposed to evaluate the impact of intragastric exposure to cigarette smoke condensate (CSC) on the development of intestinal inflammation, colitis and alterations in Paneth cells integrity in mice. For that, C57BL/6 mice received 400µg CSC or vehicle intragastrically (n=6 per group), 3 times a week for 2 weeks. After 3 weeks, ileum samples were obtained for histopathological analysis, evaluation of PCs integrity by TEM and AB-PAS staining, and quantification of its bactericidal peptides expression by qRT-PCR. In addition, mice susceptible to developing inflammatory bowel disease (IL-10-/-) were treated with CSC or vehicle (n=3) to evaluate the development of colitis. Wild type mice treated with CSC exhibited signs of ileal and colonic inflammation; normal PCs in terms of distribution and number of granules, with slight morphological changes in the granules appearance; and reduced expression of cryptdin-1 and cryptdin-4 compared to the vehicle group. IL-10-/- mice treated with CSC exhibited more signs of colitis, even in specific pathogen-free conditions. Therefore, our results show that arrival of cigarette components to the mucosa induces ileal and colon inflammation, which can be partly explained by an altered functionality of Paneth cells. Moreover, this treatment induced colitis in susceptible mice, as would occur in patients affected with CD. Further investigation is required to understand the molecular pathways involved.

Analysis of intestinal lymphoid populations in BALB/c mice underwent moderate exercise

Angel Joel Hernández-Urbán[1], Marycarmne Godínez-Victoria[1], Maria Elisa Drago-Serrano[2], Aldo Arturo Resendiz-Albor[1], Giselle Quezada Ortega[1]

[1]Escuela Superior de Medicina (IPN), Mexico City, Mexico
[2]Universidad Autónoma Metropolitana, Mexico City, Mexico
joelhdez_0119@live.com; maric_27@yahoo.com; dragome@yahoo.com; alrealdo@yahoo.com; qogiselle@outlook.com

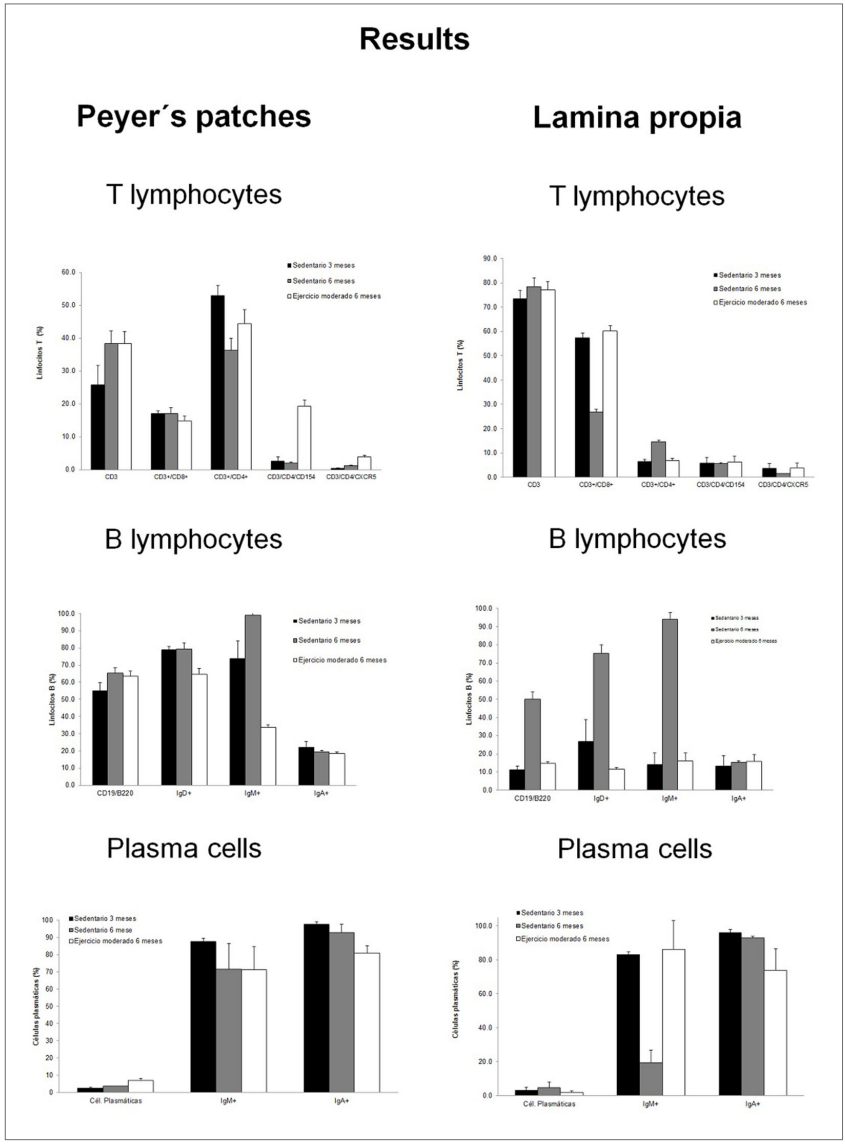
Keywords: lamina propria, Sedentary, Peyer's Patches dendritic cells, Excercise moderate, intestinal lymphoid populations

Introduction: moderate exercise displays modulatory effects on immunity that depend on intensity (moderate vs strenuous), length (short and long-term) and frequency (repeated vs occasional). During aging, a natural process of immune senesce is ameliorated by the moderate exercise. Moderate exercise restores cellular and humoral components of immune response to those found in young age.

Justification cellularity of lymphoid populations in adult age under moderate exercise is not fully known. This study may provide experimental insights on mechanisms through moderate exercise modulates the intestinal immunity.

Objective: to analyze the intestinal lymphoid T and B populations in mice underwent moderate exercise.

Material and Methods: experiments were conducted in six weeks-age old male BALB/c mice allocated in groups of eight members each as following: moderate exercised group underwent treadmill running for 6 months, young (3 months-age) and adult (6 months-age) sedentary mice control groups. Mice were sacrificed, and the small intestine dissected to prepare cell suspensions from Peyer´s patches and lamina propria. Both T and B cells populations were analyzed by cytofluorometric assays. Percentage (%) of lymphoid populations from each group were compared with one-way Anova and significant differences were regarded at $P < 0.05$.



Results: in Peyer´s patches, exercised group had greater % of T follicular helper cells [CD3+/CD4+/CXCR5+], lower % of IgD and IgM+ B cells than sedentary mice groups;

% of T helper [TCD3+/CD4+] cells in moderate exercise mice group was similar as seen in the sedentary young group. Percentage of IgM+ and IgA+ plasma cells showed no differences among mice groups. In lamina propria % of T cytotoxic, T helper lymphocytes were found similar in mice underwent moderate exercise and the sedentary young mice. In regard B populations and plasma cells moderate exercise mice had similar % as the sedentary young group by except lower % of IgA+ plasma cells.

Discussion moderate exercise may enhance the gut immunity in adult age by upmodulating T follicular helper cells in Peyer´s patches while maintaining the other lymphoid populations at levels found in young age; downmodulating effect of moderate exercise in lamina propria IgA+ plasma cells suggests a role in expression of molecules involved in gut homing via neuroendocrine factors.

Conclusions: future assays may disclose molecular neuroendocrine mechanisms underlie the impact of moderate exercise in lymphoid populations at intestinal level.

The diminution of the alergic sensitization by a probiotic and a synbiotic is vinculated to changes in the intestinal mucosa in asthmatic rats

Daniel Cervantes-García[1], Alicia Hernández-Mercado[2], Leslie Santoyo-Payan[2], Mariela Jimenez[2], Berenice Barrón-García[2], José Luis Moreno Hernández-Duque[2], Eva Salinas[2]

[1]National Council of Science and Technology – Autonomous University of Aguascalientes, Aguascalientes, Mexico

[2]Autonomous University of Aguascalientes, Aguascalientes, Mexico

cervantes.daniel@gmail.com; alize_nk@hotmail.com; leslie_san@live.com.mx; mayojv@gmail.com; berani22_7@hotmail.com; dquim2018@gmail.com; emsalin@correo.uaa.mx

Keywords: Asthma, Prebiotics, Probiotics, SCFAs, Mucosal Barrier Integrity

Allergic asthma is a heterogeneous disease in which lower airways are compromised by a chronic inflammation, which progressively diminishes the functional capacities of the lungs. There is strong evidence that gut microbiota appears to influence on allergic diseases in early-life. Microbiota diversity and dysbiosis are associated with the increased risk to develop asthma. On the other hand, prebiotics have been defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". Due prebiotic consumption can select certain bacteria, they have been used to promote the colonization or activity of probiotics in the gut and to obtain therapeutic effects. In this study, we analyzed whether the co-administration of a probiotic (Ll) alone or combined with a milk derived-prebiotic (MDP) could decrease the immune response related to allergic asthma in rats and if this effect was associated with changes in the intestinal mucosal environment. Male Wistar rats were sensitized and nebulized with ovalbulmin (OVA). Asthmatic rats were orally treated with Ll (A-LI) or Ll + MDP (A-LI/MDP) since three days prior sensitization to the end of the study. A group of asthmatic rats were orally administered with water (A) and another group was budesonide nebulized 30 min after last bronchial challenge with OVA (A-Bude). Bronchoalveolar lavage fluid (BALF) was analyzed to determine total and differential cellularity. OVA-specific IgE was quantified in serum by ELISA. Peyer's patches in small intestine were quantified and measured. Short chain fatty acids (SCFAs) were evaluated through gas chromatography. Expression of genes related to mucosal barrier integrity was analyzed by qPCR. Leucocytes infiltration in the lung was significantly reduced in 57.32% when OVA-induced asthmatic animals were treated with Ll, although this effect was not improved with the co-administration of MDP, since similar reduction

(53.94%) was achieved; as expected, budesonide nebulization reduced cells infiltration in a 65.79%, with no significant difference to A-Ll or A-LI/MDP groups. In the A group, the pulmonary inflammation was characterized by eosinophilia, which was diminished in the group A-Ll in a 68.05%, although it was only 39.76% reduced when Ll and MDP were co-administered. Besides, neutrophils and monocytes infiltration was ameliorated in the BALF of A-Ll/MDP rats, as reduction of 49.26 and 66.38% was observed, which was similar to budesonide treatment (49.03% and 60.12%, respectively). The sensitized status was tested by determination of OVA-specific IgE levels. A-Ll and A-LI/MDP groups showed significant diminution of allergen-specific IgE levels in 21.24 and 18.28%; unsurprisingly budesonide did not lessen the grade of sensitization. Since oral treatments down-regulated the inflammation and sensitization in asthmatic rats, we wanted to evaluate the mucosal barrier integrity in the gut and the production of SCFAs. Peyer´s patches had a tendency to growth in the group A-Ll in a 13.91%, however significant increment of the area was observed when Ll and MDP were co-administered since Peyer´s patches appeared 37.45 and 20.67% bigger than A and A-Ll groups. The quantity of Peyer´s patches was only increased in the A-Ll group (1.44-fold). These results indicate that after treatment of asthmatic rats with Ll and MDP the preexistent lymphoid tissue in gut increased its size more than appeared new ones. The production of SCFAs was determined in the cecal content of experimental groups. The probiotic Ll induced a discrete increment of 1.33-fold in the production of acetic acid, but when MDP was co-administered the amount of this SCFA was 2.35- and 1.76-fold higher than in A and A-Ll groups, respectively. Butyric acid levels were not significantly increased in the group A-LI/MDP when compared to A and A-Ll groups. Gene expression of IgA, MUC-2, claudin and occludin was assessed in gut tissue. The IgA expression was increased 2.60- and 2.33-fold in A-Ll and A-LI/MDP groups when compared to A group ($p < 0.05$ and $p>0.05$, respectively). The MUC-2 expression had a significant increment of 2.83 fold in A-Ll group when compared to A, although this increment was not maintained when MDP was co-administered. Besides, expression of claudin and occludin genes was significantly increased 2.47- and 4.09-fold in A-LI/MDP group, respectively, when compared to A; furthermore, when compared to A-Ll, claudin and occludin expression was 1.40- ($p>0.05$) and 1.94-fold ($p < 0.01$) higher in A-Ll/MDP group. In conclusion, the diminution of cellular and humoral markers of asthmatic rats treated with Ll, alone or in combination with MDP, is accompanied by improvement in barrier function of intestinal mucosa.

Lack of Macrophage Migration Inhitory Factor (MIF) Attenuates inflammation and clinical signs of experimental dry eye disease

Alicia Vazquez Mendoza

Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico
aliciavm@ired.unam.mx

Keywords: dry eye, MIF, inflammatory mediators, Tear film, ocular surface inflammation

Pablo E. Hernández 1, José L Reyes2, Oscar A. Ramos1 Imelda Juárez 3, Miriam Rodríguez3 and AliciaVázquez1.
1 Laboratorio de Inflamación Ocular, Carrera de Optometría FES Iztacala, Estado de México, México. 2Laboratorio de Inmunología Experimental y Regulación de la Inflamación Hepato-Intestinal, UBIMED, FES Iztacala, Estado de México, México. 3Laboratorio de Inmunidad Innata UBIMED, FES Iztacala, Estado de México, México.

Dry eye disease (DED) is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, such as tear film instability, hyperosmolarity, ocular surface inflammation and neurosensory abnormalities play etiological roles (DEWS 2017). The immunopathogenesis of DED is not yet fully understood, but ocular surface inflammation has been shown to play a significant role in the development and progression of DED. Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine produced by pituitary gland and different immune cell types including macrophages (Mo), dendritic cells (DC) and T cells. MIF induces the expression of other inflammatory mediators, such as TNF-alfa, nitric oxide (NO), cyclooxygenase 2 (COX2). To determine the role of MIF on inmunopatogenésis of DED we used male knockout (KO) and wild-type (WT) BALB/c mice. Seven to 8-week-old BALB/c mice were housed in a controlled environment chamber and were treated with subcutaneous scopolamine (0.5mg-three times a day) for 21 days to induce DED. Tear volume, fern ing pattern, conjunctival globet cells density and inflammatory cytokines were measured by schirmer and fern ing test, impression cytology and ELISA-sandwich respectively. Following DED induction, we found that wild-type mice had significantly decreased in tear production, number of goblet cells and abnormal fern ing patterns (types III or IV) and increased levels of TNF-alfa in serum. In contrast, mice lacking MIF preserved globet cells and mucin panthers. Histological analysis revealed more inflammatory infiltrated in lacrimal gland from WT mice as compared to MIF knockout (KO). Our data suggest that MIF has a key role in the development of DED through induction of inflammatory mediators which provides another therapeutically target in DED.

Polysaccharides as target of thymus-independent mucosal immune responses and possible cross protection

Miriam SJB Lastre González[1], Laura María Reyes Díaz[1], Yusnaby Borrego[1], Raúl Ramos Pupo[1], Victoria Ester González Ramírez[1], Irma Gudelia Vega García[1], Dainerys Aleidad Pérez Lastre[2], Martha Leonor Paradoa Pérez[1], Alexander Batista Duharte[3], Carlos Bauman[4], Roberto R Grau[4], Oliver Pérez[1,5]

[1]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[2]Curitiba, Curitiba, Brazil
[3]Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista Júlio de Mesquita Filho, Araraquara, Brazil
[4]National University of Rosario, Rosario, Argentina
[5]Escuela Latinoamericana de Medicina, La Habana, Cuba
oliver.perez@infomed.sld.cu; lreyes@giron.sld.cu; ybarrogo@giron.sld.cu; raulramos@infomed.sld.cu; victoria.gonzalez@infomed.sld.cu; irmav@giron.sld.cu; dperezlastre@gmail.com; mparadoa@infomed.sld.cu; batistaduhartea@gmail.com; carlosbauman@gmail.com; robertograu.conicet@gmail.com; oliverperez@giron.sld.cu

Keywords: Polysaccharides, Vaccines, mucosal immunity, Crossprotection, thymus-independent

Introduction: Polysaccharides (Ps) are virulent factors of encapsulated bacteria used for serotypic classification. Ninety four Streptococcus pneumoniae, 13 Neisseria meningitides, and several Haemophilus influenza (where the b (Hib) is the most pathogenic) serotypes have been documented [1,2,3]. Several plain Ps vaccines against these bacteria were developed. Ps are thymus-independent type 2 antigens (TI-2)4 that stimulate directly B lymphocytes eliciting IgM with limited or absence of class switching, affinity maturation, and immunological memory5. They are immunogenic, provide protection in healthy adults and reduce the risk of invasive disease6, but they have low immunogenicity and lack of booster responses in children younger than 2 years of age7,8. The covalent conjugation to a carrier protein overcomes these problems inducing specific IgG, long-lived plasma cells and memory B cell development9. Secretory IgA (SIgA) is the main mucosal protective antibody. Plain and conjugated Ps vaccines are used alone or with additional adjuvants by parenteral route10 but without induction of mucosal response. Nevertheless, microorganisms arrive and establish at mucosal route. Therefore, what is the relationship of the immune response induce by vaccination vs. infection or commensals?

Aims: To compare some immune responses induce by polysaccharides that arrived naturally, with un-conjugated, or conjugated vaccines.

Results: There are 28 different serotypes (divalent N. meningitides serogroups A and C; four valences N. meningitides serogroups A, C, W135, and Y; Typhoid Vi; and 23-valent pneumococcal) covered by plain Ps vaccines and 12, 15 or 18 different serotypes (Hib, tetravalent N. meningitides serogroups A, C, W135, Y, 7(10 or 13)-valent pneumococcal, and poliovirus) covered by covalent conjugated vaccines10. All these vaccines are applied by parenteral (intramuscular or subcutaneous) route. Pneumococcal vaccines significantly reduce invasive diseases11,12, but coverage is limited to the vaccine serotypes including carriage stages but an emergence of non-vaccine replacement serotypes1,11,13–15 were observed and the induction of significant SIgA was not deeper looking. However, a rapid induction and decline of serum monomeric IgA after vaccination was observed. Fast response to capsular serotypes suggests that this isotype is produced by an innate-like B cell population in humans16. Natural acquisition of nasopharingeal IgA mucosal antibody over time was observed and colonized-children by S. pneumoniae had higher mucosal antibody levels than noncolonized children17. The specific SIgA responses to 23-valent pneumococcal Ps vaccine was found in the saliva of children immunized at age 13 months after priming with 3 doses of conjugate vaccine in early infancy18. Induction of functional SIgA responses in breast milk of pneumococcal vaccinated mother was observed19. In addition, in the absence of immune deficiency (like complement components that increased ~1000 times Neisseria infection), disease caused by Ps bacteria occurs only one time in live; but carries stage follows acute, transient, or chronic patters. These arrive by mucosa route and the induction of specific SIgA occurs. In this sense higher naturally acquired mucosal antibody levels to some pneumococcal proteins are associated with reduced acute otitis media caused by S. pneumoniae17. Furthermore, studies with the Cuban bivalent vaccine (VA-MENGOC-BC®), which contains non-covalently conjugated serogroup C Ps (PsC), showed that teenagers vaccinated in their infant life after a third dose or to natural Neisseria challenge induced a significantly IgG anti PsC response20. This vaccine did not induce mucosal specific SIgA response; but could boost the specific IgA memory lymphocyte induced by natural infection/carriage in human. In mice, nasal immunization of AFCo1 (Adjuvant Finlay Cochleate 1 derived from AFPL®1)21–25 co-administered with PsC induces mucosal and systemic anti PsC immune responses26. In addition,IgA is produce as TI and TD (dependent) manner27,28. The former is mainly induced by Ps29-32. Consequently, IgA is more cross reactive than IgG and it could be induced by microbiota33.

Conclusions: Only mucosal route of natural infection or vaccination induce enough SIgA to help in the protection of the mucosal system. The induction of polysaccharide crossprotection by mucosal vaccination should to be achieved with mucosal adjuvants which are not yet available.

REFERENCES

1. van der Linden M, Reinert RR, Kern WV, Imohl M. Epidemiology of serotype 19A isolates from invasive pneumococcal disease in German children. BMC infectious diseases. 2013;13:70.10.1186/1471-2334-13-70.
2. Calix JJ, et al. Biochemical, genetic, and serological characterization of two capsule subtypes among Streptococcus pneumoniae Serotype 20 strains: discovery of a new pneumococcal serotype. J Biol Chem. 2012; 287:27885-27894.
3. Poolman JT et al. Serotypes and Subtypes of Neisseria meningitides: Results of an International Study Comparing Sensitivities and Specificities of Monoclonal Antibodies. Clinical and Diagnostic Laboratory Immunology 1995; 2(1):69-72.
4. Weintraub A. Immunology of bacterial polysaccharide antigens. Carbohydr Res (2003) 338:2539-47. doi:10.1016/j.carres.2003.07.008.
5. Jakobsen H, Jonsdottir I. Mucosal vaccination against encapsulated respiratory bacteria – new potentials for conjugate vaccines? Scand J Immunol 2003; 58:119-28. doi:10.1046/j.1365-3083.2003.01292.
6. Jackson LA, Neuzil KM. Pneumococcal polysaccharide vaccine. 5th ed. In: Plotkin SA, Orenstein WA, Offit PA editors. Vaccines. Philadelphia: Saunders (2008). p. 569-604.
7. Pollard AJ, Perrett KP, Beverley PC. Maintaining protection against invasive bacteria with protein polysaccharide conjugate vaccines. Nat Rev Immunol 2009; 9:213-20. doi:10.1038/nri2494 84.
8. Konradsen HB. Quantity and avidity of pneumococcal antibodies before and up to five years after pneumococcal vaccination of elderly persons. Clin Infect Dis (1995) 21:616-20. doi:10.1093/clinids/21.3.616
9. Poland GA. Prevention of meningococcal disease: current use of polysaccharide and conjugate vaccines. Clin Infect Dis (2010) 50(Suppl 2):S45–53. doi:10.1086/648964
10. Pérez O, Batista-Duharte A, González E, Zayas C, Balboa J, Cuello M, Cabrera O, Lastre M and Schijns VEJC. Human prophylactic vaccine adjuvants and their determinant role in new vaccine formulations. Braz J Med Biol Res, 2012; 45(8):681-692. doi: 10.1590/S0100-879X2012007500067.
11. Lynch JP. 3rd, Zhanel GG. Streptococcus pneumoniae: epidemiology and risk factors, evolution of antimicrobial resistance, and impact of vaccines. Curr Opin Pulm Med. 2010; 16:217-225.
12. Ruckinger S, van der Linden M, Reinert RR, von Kries R. Efficacy of 7-valent pneumococcal conjugate vaccination in Germany: An analysis using the indirect cohort method. Vaccine 2010; 28:5012-5016.
13. Besser TE, McGuire TC, Gay CC, Pritchett LC. Transfer of functional immunoglobulin G (IgG) antibody into the gastrointestinal tract accounts for IgG clearance in calves. Journal of virology 1988; 62:2234-2237.
14. Pichichero ME, Casey JR. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. Jama. 2007; 298:1772-1778.
15. Kaplan SL, et al. Serotype 19A Is the most common serotype causing invasive pneumococcal infections in children. Pediatrics. 2010; 125:429–436.10.1542/peds.2008-1702.
16. Crowther RR et al. Rapid kinetics of serum IgA after vaccination with Prevnar*13 followed by Pneumovax*23 Heliyon. 2017; 3(2): e00255. doi: 10.1016/j.heliyon.2017.e00255

17. Xu Q, Casey JR, and Pichichero ME. Higher Levels of Mucosal Antibody to Pneumococcal Vaccine Candidate Proteins Are Associated with Reduced Acute Otitis Media Caused by Streptococcus pneumoniae in Young Children. Mucosal Immunol. 2015 September ; 8(5): 1110–1117. doi:10.1038/mi.2015.1.

18. Choo S, Zhang Q, Seymour L, Akhtar S, Finn A. Primary and booster salivary antibody responses to a 7-valent pneumococcal conjugate vaccine in infants. J Infect Dis 2000; 182:1260-3.

19. Finn A et al. Induction of Functional Secretory IgA Responses in Breast Milk, by Pneumococcal Capsular Polysaccharides J Infec Dis 2002; 186:1422-9.

20. Pérez O, Romeu R, del Campo J, Zayas C, Lastre M. Proteoliposome and polysaccharide-based meningococcal vaccine are immunogenic in infants and toddlers and primes for memory against serogroup C polysaccharide. WJV World Journal of Vaccines 2013; 3(2):77-87. doi:10.4236/wjv.2013.32012.

21. Pérez O, Bracho G, Lastre M, del Campo J, Gil D, Zayas C, Acevedo R, González D, López JA, Taboada C, Solis RL. Novel adjuvant based on a proteoliposome-derived cochleate structure containing native lipopolysaccharide as a pathogen-associated molecular pattern. Immunology and Cell Biology 2004; 82,603-610.

22. O Pérez, G Bracho, M Lastre, C Zayas, D Gonzólez, D Gil, J del Campo, R Acevedo, C Taboada, T Rodríguez, ME Fajardo, G Sierra, C Campa, N Mora, R Barberá, and RS Solís. Proteliposome-derived Cochleate as an immunomodulator for nasal vaccine. Vaccine 24(S2):52, 2006.

23. Pérez O, Lastre M, Cabrera O, del Campo J, Bracho G, Cuello M, Balboa J, Acevedo R, Zayas C, Gil D, Mora N, González, D, Pérez R, González E, Barberá R, Fajardo EM, Sierra G, Solís RL, and Campa C. New vaccines require potent adjuvants like AFPL1 and AFCo1. Scand J Immunology 2007; 66:271-77.

24. J del Campo et al. Intranasal immunization with a proteoliposome-derived cochleate containing recombinant gD protein confers protective immunity against genital herpes in mice. Vaccine 2010; 28(6):1193-1200.

25. Romeu B, González E, Zayas C, del Campo J, Acevedo R, Cuello M, Valdes Y, Balboa J, Cabrera O, Lastre M, Pérez O. AFCo1 as nasal adjuvant of capsular polysaccharide from Neisseria meningitidis serogroup C induces systemic and mucosal immune responses. Scand J Infect Dis (2011) 43:809-13. doi:10.3109/00365548.2011.586648.

26. Pérez O, Romeu B, Cabrera O, González E, Batista-Duharte A, Labrada A, et al. Adjuvants are key factors for the development of future vaccines: lessons from the Finlay adjuvant platform. Front. Immunol. 2013; 4:407:1-12. doi: 10.3389/fimmu.2013.00407.

27. Macpherson AJ, et al. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 2000; 288:2222-2226.

28. Cerutti A. The regulation of IgA class switching. Nature Reviews Immunology 2008; 8:421-434. doi:10.1038/nri2322).

29. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 1981; 68:356-364.

30. Reyes Díaz LM. PhD thesis. Instituto de Ciencias Básicas y Preclínicas "Victoria de Girón", Universidad de Ciencias Médicas de la Habana, 2018.

31. Bunker JJ, et al. Natural polyreactive IgA antibodies coat the intestinal microbiota. Science 2017; 20;358(6361). DOI: 10.1126/science.aan6619.

32. Pérez O, Reyes Díaz LM, Batista-Duharte A, Ramos Pupo R, Pérez Nicado R, Borrego Y, Pérez Lastre D; Grau RR, and Lastre González MSJB. Challenging the adjuvant´s Gaston definition. Frontier in Immunology. ImmunoCancun 2018 abstract.

33. Bunker JJ, et al. Natural polyreactive IgA antibodies coat the intestinal microbiota. Science 2017; 20;358(6361). DOI: 10.1126/science.aan6619.

Interleukin 6 activates STAT3 in colonic intestinal epitelial cells

Octavio López Méndez, Carolina Ivonne Serrano García, José Fernando Rubio Ramírez, Aurora Candelario Martínez, Carlos Samuel Galan Enriquez, Sandra Denisse Chanez Paredes, Vianney Ortiz-Navarrete, Michael Schnoor, Porfirio Nava Domínguez

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico

lopezoctavio.itsr@live.com.mx; ivonne40_45@hotmail.com; fhernandorubio@gmail.com; aurorac@fisio.cinvestav.mx; cgalane@cinvestav.mx; schanez@gmail.com; vortiz@cinvestav.mx; mschnoor@cinvestav.mx; pnavaster@gmail.com

Keywords: Homeostasis, Interleukin 6, stat3, epitelial cells, bowel inflamation

The transcription factor STAT3 plays a mayor role in several autoinmune and cancer pathologies. In the Intestinal epithelium have been shown that STAT3 plays a critical role in intestinal homeostasis and mucosa regeneration after inflamation insult. One of the upstream signal identifed to stimulate STAT3 activation is interleukin 6.Whitin the intestine, IL-6 has been shown to prevent apoptosis during prolongate bowel inflamation. Here we evaluated the interleukin 6 efect on STAT3 using a murine model of colon inflamation induced by Dextran sulfate sodic. We demostrated that STAT3 is activated progresivelly in epitelial cells during inflamation progression. Also, we observed that IL-6 stimul by intraperitoneal injection actívate STAT3. Our data demostrate an important role of interleukin 6 in the epitelial cells during a inflamation insult.

STAT signaling in Intestinal Epithelial Cells during colitis

José Fernando Rubio Ramírez[1], Carolina Ivonne Serrano[2], Octavio López Méndez[2], Aurora Candelario Martínez[2], Carlos Samuel Galán Enríquez[2], Sandra Denisse Chanez Paredes[2], Vianney Ortiz-Navarrete[2], Michael Schnoor[2], Porfirio Nava Domínguez[2]

[1]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
fhernandorubio@gmail.com; ivonne40_45@hotmail.com; lopezoctavio.itsr@live.com.mx; aurorac@fisio.cinvestav.mx; cgalane@cinvestav.mx; schanez@gmail.com; vortiz@cinvestav.mx; mschnoor@cinvestav.mx; pnava@fisio.cinvestav.mx

Keywords: Inflammatory Bowel Diseases, IFN-gamma, IL-6, Ulcerative colitis (uc), STAT1/3 signaling pathway

Intestinal inflammatory diseases (IBD) encompass a group of pathologies with similar pathophysiology and symptomatology, but with an etiology not yet elucidated. Crohn's Disease (CD) and Ulcerative Colitis (UC) are the main representatives of IBD.

Until now is well accepted that not a single factor can positioned as the trigger that induces IBD development. However, it is well known that disruption of the intestinal barrier can leads to a series of events that exacerbate the innate and adaptive immune response in the colon. This process triggers multiple biological effects, including cytokine release which has been linked to IBD development. Thus disruption of the intestinal epithelial barrier and leakage of antigens directly contributes to IBD development and establishment. However the mechanisms underlying such process are not well defined.

Interferon gamma (IFN gamma) and Interleukin 6 (IL-6) are proinflammatory cytokines that play an important role in the regulation of intestinal epithelial cells (IEC) homeostasis during IBD by modulating STAT signaling. Therefore in this work we evaluated the effect of IFN gamma and IL-6 in the activation of its downstream targets, STAT1 and STAT3 respectively.

SW480 cells were cultured in plate dishes until 80% of confluence and treated for 30 minutes with IL-6 or IFN-gamma. Then SW480 cells were treated with cytokines or BSA for 0,0.5, 1, 2,4 hours and after that media was removed, collected and total adhesive cell lysed in RIPA buffer. Evaluation of secreted proteins was performed

by Elisa and the status of STAT proteins was analyzed by westernblotting using specific phosphoantibodies. Unexpectedly we observed that IFN gamma activates Stat1 and Stat3 signaling in IECs meanwhile IL-6 treatment induces activation of STAT3. Interestingly, activation of STAT3 in IECs that was IFN gamma -mediated depends on the secretion of IL6 by the epithelial cells. Furthermore, in agreement with these results we observed activation of STAT3 in mucosal cell lysates obtained from WT mice that were administered intraperitoneally with IFN gamma. IL-6 intraperitoneal injection in contrast only activates STAT3 in the colonic cell tissue. Finally we also observed STAT3 and STAT1 activation in the colonic mucosa of WT mice treated with DSS to induce colitis. However, meanwhile STAT3 accumulates in the nuclear compartment of IECs along the whole crypt axis no clear staining for STAT1 was observed.

Thus in conclusion these results suggest that IFN gamma and IL6 can activate STAT3 in the intestinal epithelium during inflammation.

Effect of cholecystokinin (CCK) on the production of IgA in the small intestine of mice

Juan Morales Magaña, Ivonne Arciniega Martínez, Aldo A. Resendiz Albor, Rosa A. Jarillo Luna, Rafael Campos Rodríguez, Edgar J. Rodriguez Rodriguez, Judith del C. Pacheco- Yepez

Instituto Politécnico Nacional, Mexico City, Mexico
blue-tab@hotmail.com; ivonne.arciniega.77@gmail.com; ; alrealdo@yahoo.com.mx; roadjalu12@gmail.com; citlicampos@gmail.com; jinzo27300@gmail.com; payejuca@prodigy.net.mx

Keywords: Cholecystokinin, Inflammation, Obesity, intestine, satiety

The gastrointestinal tract is the largest endocrine organ in the body, containing hundreds of thousands of cells that react to changes in the intestinal content by secreting hormones, including cholecystokinin (CCK). This hormone is a linear polypeptide that exists in multiple molecular forms, the octapeptide; CCK-8 is the classic cerebral and intestinal form. It is secreted in the duodenum in response to food, the main stimuli for its secretion are fats and proteins. In the intestinal mucosa several mechanisms have been developed to neutralize pathogens and antigens of the diet, the synthesis of IgA is a key strategy to generate immunoprotection in the absence of inflammation, so this antibody is critical for survival of the species.

IgA in dimeric form is transported to the intestinal lumen attached to the Ig polymeric receptor (pIgR) as secretory IgA (SIgA). There is little information about the "Diet-CCK-SIgA" relationship. Recently it has been reported that the presence of proteins increases the secretion of duodenal IgA and this effect was blocked when administering a CCK antagonist, which indicates that at least in part CCK is responsible for the increase of IgA. The purpose of the present study was to elucidate the effect of CCK on IgA secretion. For this, 6 and 8 μg/kg were administered as a single dose of CCK-8 to male BALB/c mice, which were sacrificed 2 hours later to obtain intestinal fluid in which the concentration of total IgA and SIgA was quantified by ELISA's, lymphocytes of the lamina propria were obtained to determine the percentage of populations of B lymphocytes (IgM+, IgA+) and plasma cells (CP) IgA+ by flow cytometry. The results show reduction of the total IgA concentration and SIgA with both doses of CCK. The reduction in total IgA was dependent on the concentration. On the other

hand, decrease in B lymphocytes IgA+ (9.1%) and IgM+ (20.7%) was observed with respect to the control animals (61% and 27.5%, respectively); and lower percentage of CP IgA+ (9%) compared to the control group (16.15%). The results suggest that CCK regulates the intestinal immune response through the modification of IgA secretion.

Supported by COFAA-IPN.

IFN-gamma regulates STAT3 activation IL-6 mediated in epithelial cells during colitis

Carolina Ivonne Serrano García, José Fernando Rubio Ramírez, Octavio López Méndez, Aurora Antonia Candelario Martínez, Carlos Samuel Galan Enriquez, Sandra Denisse Chanez Paredes, Vianney Ortiz-Navarrete, Michael Schnoor, Porfirio Nava Domínguez

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico

ivonne40_45@hotmail.com; fhernandorubio@gmail.com; lopezoctavio.ITSR@LIVE.COM.MX; aurorac@webmail.fisio.cinvestav.mx; cgalane@cinvestav.mx; schanez@gmail.com; vortiz@cinvestav.mx; mschnoor@cinvestav.mx; pnava@fisio.cinvestav.mx

Keywords: Colitis, IFN-gamma, IBD, IL-6, STAT3 pathway

Inflammatory bowel diseases (IBD) is a group of intestinal disorders cause for chronic inflammation. Crohn´s disease and ulcerative colitis are the two most common diseases. The physiopathology of IBD is unknown; however, the immune system and the local production of cytokines have been associated with the progression of the disease.

INFg is a proinflammatory cytokine critically for IBD development. This cytokine increases during colitis, and plays an important role in the development of chronic inflammation in the colon and other organs. IL-6 is other cytokine that has been implicated in the regulation of crypt homeostasis during inflammation through the activation of STAT3. In this work, we evaluated the role of IFNg in the regulation of the IL-6/STAT3 signaling in intestinal epithelium during colitis.

To examine the STAT3 activation during colitis we used the murine dextran sulfate sodium (DSS) colitis model of acute epithelial injury and mucosal inflammation. In agreement with previous published **results** we observed activation of STAT3 in IECs throughout the entire crypt beginning 4 days after treatment and this activation correlated with an increase in the IL-6 levels present at the mucosal tissue. To corroborate that STAT3 activation in intestinal epithelium during colitis is due to cytokine increase, we treated animals with IL-6 or IFN . Immunofluorescence analysis showed that the intraperitoneal administration of IL-6 does not activate STAT3 in epithelial cells, however IFN administration activate STAT3 in the colonic epithelium. This suggests that IFN may be responsible for the activation of STAT3 in IECs during colitis.

To evaluate the mechanism by which IFNg induces STAT3 activation in IECs we treated SW480 cells with IL-6 or IFNg for 30 min, followed by washout, and measured STAT3

phosphorylation at 30 min, 1h, 2h y 4h. The analysis showed that STAT3 phosphorylation is maintained after cytokines have been removed, suggesting that both cytokines could not only activate STAT3 directly, but could also induce the secretion of factors that maintain the STAT3 phosphorylation. Furthermore, conditioned medium from IL-6 or IFNg-treated cells induces STAT3 activation and no STAT3 activation was detected in cells exposed to IL-6 or IFN that were pretreated with Brefeldin A. These findings support the notion that STAT3 activation in epithelial cells is mediated by the secreted factor after cytokine stimulation. The analysis of the conditioned media obtained from isolated mouse crypts or SW480 demonstrated that both cytokines, IL-6 and IFNg, increased the secretion of epithelial IL-6 and this increase is responsible of STAT3 activation.

Finally, our results demonstrated that epithelial cells obtained from colon of DSS treated mice can secrete high amounts of IL-6 that could be responsible for the activation of STAT3. Thus, taken together these results demonstrated that IFNg plays an important role in the maintenance of epithelial cell homeostasis during colitis by positively regulating the activation of IL-6/STAT3 signaling.

Effects of stimulation of muscarinic receptors in the secretion of IgAs intestinal in mice

Xochitl Abril Rebollar Ruíz, Aldo Arturo Resendiz Albor, Ivonne Maciel Arciniega Martinez, Mónica Miguel Santamaría Chavez, Rafael Campos Rodriguez

Instituto Politécnico Nacional, Mexico City, Mexico
xarebollarr@gmail.com; alrealdo@yahoo.com.mx; ivonne.arciniega.77@gmail.com; yaestabaese@gmail.com; citlicampos@gmail.com

Keywords: Atropine, Muscarine, IgA, Mouse, Citokyne

The peripheral nervous systems through acetylcholine regulates the immune system by muscarinic and nicotinic receptors, they have effects on the activation and inactivation of immune cells and contribute to the secretion of immunoglobulins such as IgA. The aim of the present study is to evaluate the effect of stimulation of muscarinic receptors in the secretion of intestinal IgA in BALB/c mice. Total IgA concentration and secretory IgA were measured after i.p. injection with the muscarinic cholinergic agonist muscarine or the muscarinic cholinergic antagonist atropine, and IgA+ plasma cells of lamina propria and intracelular cytokines TGF-β, IL-4, IL-5, IL-6 e IL-10 related with via T- dependent for isotype switching were measured by flow citometry. Our results show that in muscarine and atropine group the secretory IgA remained unaltered, the concentration of total IgA has decreased in atropine group, but this remain unaltered in muscarine group. IgA+ plasma cells, IL-4, IL -5, IL-10 of lamina propia had an increase of the muscarine group. All the citokines decreased in atropine group, principally TGF-β. It appears that the muscarinic receptors modify the secretion of IgA, it necessary to continue investigating to elucidate the way in which acetylcholine regulates the secretion of IgA in the mice intestine.

High fat diet feeding modifies experimental dry eye disease in a strain-dependent manner

Jose Luis Reyes

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
jlreyes@ired.unam.mx

Keywords: Inflammation, Obesity, dry eye, high fat diet, Ocular surface

*Evelin G. Morales1, Alicia Vázquez-Mendoza2, Danielle T. Vannan3, Bertus Eksteen4, Marisol I. González1, Jazmín Perez1, Luis I. Terrazas5 and José L. Reyes1**
1Laboratorio de Inmunología Experimental y Regulación de la Inflamación Hepato-Intestinal, UBIMED, FES Iztacala, Estado de México, México. 2Laboratorio de Inflamación Ocular, Carrera de Optometría, FES Iztacala, Estado de México, México. 3Snyder Institute for Chronic diseases, University of Calgary, Calgary AB, Canada. 4Aspen Woods Clinic, Calgary AB, Canada. 5Laboratorio de Inmunoparasitologia, UBIMED, FES Iztacala, Estado de México, México.

Background: Inflammatory diseaseas result from a complex interplay between individuals` genome and environmental factors that either allow or alter the development of such diseases. Simultaneous efforts are taking place to identify genes that increase susceptibility to inflammatory processes as well as the role of a variety of environmental factors (infections, sleep deprivation, sunlight exposure, smoking and diet) on immunopathology of autoinflammatory and autoinmune disease. Therefore, environment has emerged an important driver that ultimately may affect several stages of disease like onset, severity and treatment response. In regards of these environmental factors, diet-induced obesity has recently received much attention since its increasing incidence parallels heightened presence of inflammatory diseases and it is thought that increasing global rates of obesity are affecting the development of many immune-mediated diseases. Thus, understanding the underlying mechanisms through which obesogenic diets alter inflammatory responses may pave the way to identify novel therapeutic targets. Ocular diseases represent a challenge given the intrinsic complexity of such organ. The impact of high fat diet (HFD)-induced obesity on ocular surface inflammation has been poorly addressed. Here, we explore how HFD-feeding changes the disease outcome on a mouse model of dry eye disease (DED) and also compare the impact of genetic background over the course of DED by using C57BL/6 and BALB/c mice strains.

Methods: We fed both mice strains (C57BL/6 and BALB/c) with the westernized-like obesogenic diet 5TJN (Test diets) for 10 weeks. In another set of experiments, we fed the mice only for 2 weeks (while the dry eye model protocol lasted). Bodyweight changes and blood glucose levels were recorded weekly. To impair lacrimal gland functions

(DED experimental model), mice received three daily subcutaneous injections of 0.5 mg of scopolamine (Sigma Aldrich) for 14 days. Also, a small fan was placed next to the cage to generate more desiccant stress. The severity of experimental DED was determined by measuring tear volume Schirmer`s test (ophthalmic strips), mucin pattern in tear samples (Ferning test) and impression cytology in order to assess loss of goblet cells.

Results: Simultaneous feeding with a HFD while DED is induced did not alter the DED outcome neither in C57BL/6 nor BALB/c strains, as gauged by smililar tear volume, mucin patterns and numbers of goblet cells in both standard chow-fed and HFD-fed experimental animals.from. Interestingly, long term feeding (10 wks) with a westernized diet results in slight modifications of experimental DED in BALB/c whereas C57BL/6 under the same feeding protocol showed significantly improved signs.

Conclusion: High fat diet feeding did not worsen experimental DED, rather improved DED outcome in a strain-dependent manner.

Antibody responses against *Saccharomyces cerevisiae*, D-mannose and D-glucose in Crohn's disease patients and non-ill subjects

Raúl Ramos Pupo[1], Miriam SJB Lastre González[1], Braulio Limas de Armas[1], Deyanira La Rosa Hernández[1,2], Laura María Reyes Díaz[1], Irma Gudelia Vega García[1,3], Yusnaby Borrego[1], Liana M Avila Salcedo[1,4], Oliver Pérez[1,3]

[1]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba

[2]Instituto de Gastroenterologia (Cuba), La Havana, Cuba

[3]Escuela Latinoamericana de Medicina, La Habana, Cuba

[4]Hospital Clìnico Quirúrgico Joaquín Albarrán. Ave. 26 esq Ave. de la Independencia, Puentes Grandes. Plaza de la Revolución, Havana, Cuba

raulramos@infomed.sld.cu; oliver.perez@infomed.sld.cu; brauliolimas@infomed.sld.cu; deyani@infomed.sld.cu; lreyes@giron.sld.cu; irmav@giron.sld.cu; yborrego@giron.sld.cu; lianavila@infomed.sld.cu; oliverperez@giron.sld.cu

Keywords: Polysaccharides, Saccharomyces cerevisiae, IgA, mucosal immunity, Chrohn disease

Crohn's disease is a chronic inflammatory disorder of multifactorial cause where certain environmental factors that affect genetically predisposed individuals, generates a dysregulation of the immune response directed to members of the intestinal microbiota that leads to the alteration of the barrier mechanisms at the digestive tract level and consequently to the chronic inflammation thereof. In a healthy intestinal lumen, there is a balance between defenses against pathogens and tolerance to microbiota. Thus, inflammatory responses coexist with predominant endogenous anti-inflammatory mechanisms. Once intestinal inflammation is activated in Crohn's disease, several phenomena are established: massive infiltration of immune cells; production of inflammatory mediators; destruction of the intestinal barrier and dysregulation of the feedback systems. Consequently, a dysbiosis occurs and the induction of extraluminal immune response against microbiota [1–4]. Serum IgG against S. cerevisiae is found in 60–70% of patients with Crohn's disease, 10–15% of patients with ulcerative colitis, and 8% of healthy controls5. However, anti S. cerevisiae serum and mainly mucosal IgA have been poorly studied in Crohn's disease and not in healthy subjects. In addition, D-mannose is the homopolysaccharide of mannan, a constituent of cell wall of this yeast. This is a MAMP (microbial associated molecular pattern) that stimulate the innate response through CLRs (C-type lectin receptors); but it is not expected to induce antibody response. D-glucose is the homopolysaccharide of b-glycan of cell

wall of this yeast, very structurally similar to D-mannose and very frequent in human diet. Therefore, it is not expect to induce mucosal IgA response. Nevertheless, antibody responses against D-mannose and D-glucose in Crohn's disease patients and non-ill subjects have not been explored yet. In this sense, the present research aims to expand new diagnostic horizons and to test hypotheses related to the mechanisms of response to antigens in this disease.

Aims: To evaluate serum IgG and salivary IgA responses against Saccharomyces cerevisiae, D-mannose and D-glucose in subjects with and without this disease.

Methods: Serum and parotid saliva samples of 49 subjects were obtained, 20 with Crohn's disease and 29 without it. Anti S. cerevisiae, D-mannose, and D-glucose serum IgG and salivary IgA responses were determined by ELISA.

Results and Discussion: Crohn's disease patients showed serum IgG positivity against S. cerevisiae, D-mannose, and D-glucose in 100; 70; and 100%, respectively; while non-ill subjects presented positivity in 3,4; 6,9; and 0%; respectively. In an opposite pattern, Crohn's disease patients showed salivary IgA positivity against S. cerevisiae, D-mannose, and D-glucose in 4; 0; and 10%, respectively; while non-ill subjects presented positivity in 41,4; 100; and 100%, respectively. (Tab) The high percentage of patients with anti S. cerevisiae serum IgG positivity is in correspondence with other autors5,6,7,8,9 and it is useful for differential diagnosis of Crohn's disease. Anti mannan serum IgA response in Crohn's disease has been also reported10,11. Nevertheless, antibody responses against D-mannose monosaccharide have not been described before. This was an amazing result as D-mannose is a MAMP that stimulates the innate but not the acquired immune response. D-mannose frequently exhibits at the end of microorganisms' polysaccharides. In contrast, it mainly keeps internally and not terminally in humans' glycans. This explains MAMP action of exogenous challenges. Anti D-glucose responses have not been described before either. This monosaccharide is very common in nature and is an epimer of D-mannose that only differs in an alcohol group position12. A contrasting pattern to that observed in the serum response occurs in mucous membranes. Salivary IgA anti S. cerevisiae, D-mannose, and D-glucose were significantly higher ($p < 0{,}001$) in non-ill subjects than in Chohn´s patients. Adequate levels of mucosal IgA would be necessary to prevent adherence and penetration of microbes that trigger transmural inflammatory response in Crohn's disease. The lower levels of salivary IgA against S. cerevisiae, D-mannose and D-glucose found in patients with this disease could be due to the dysfunction in the barrier mechanisms to microbes. This fact implicates the available IgA could be captured and occupied in

Table. IgG and IgA anti *S. cerevisiae*, D-mannose, and D-glucose positivity in Crohn's disease patients and non-ill subjects. Represented are the absolute frequencies per assays according to cutoff levels and condition of Crohn's disease. Each assay corresponded to an indirect ELISA to identify the corresponding specificity. Absolute frequencies and percentages (in parentheses) of positive (+) and negative (-) individuals according to condition of Crohn's disease are shown for each assay. The total sample was 49 individuals: 20 patients with Crohn's disease and 29 individuals without it.

Assay		Crohn's disease patients (n = 20)	Non-ill subjects (n = 29)	Total (n = 49)
Anti S. *cerevisiae* serum IgG	+	20 (100%)	1 (3,4%)	21
	-	0 (0%)	28 (96,6%)	28
Anti D-mannose serum IgG	+	14 (70%)	2 (6,9%)	16
	-	6 (30%)	27 (93,1%)	33
Anti D-glucose serum IgG	+	20 (100%)	0 (0%)	20
	-	0 (0%)	29 (100%)	29
Anti S. *cerevisiae* salivary IgA	+	1 (5%)	12 (41,4%)	13
	-	19 (95%)	17 (58,6%)	36
Anti D-mannose salivary IgA	+	0 (0%)	29 (100%)	29
	-	20 (100%)	0 (0%)	20
Anti D-glucose salivary IgA	+	2 (10%)	29 (100%)	31
	-	18 (90%)	0 (0%)	18

its antigen binding sites in submucosa itself, before its binding to poli Ig receptor and being transported unidirectionally towards the lumen. The generation of an extensive chronic proinflammatory microenvironment along the intestinal mucosa, as occurs in this disease, can contribute significantly to the observed behavior. The production of cytokines such as IL-12 and IL-23 by antigen-presenting cells and epithelial cells is essential for the generation of a predominantly Th1/Th17 pattern. The released of INF-g induces class change towards IgG and thus antagonizes the change of class towards IgA in the plasma cells placed in submucosa. In this context, the lower proportion of TGF-b, an immunoregulatory cytokine essential for the change of class to IgA, can also contribute to the reduction of specific IgA. TGF-b is produced not only by antigen-presenting cells and epithelial cells, but also by regulatory T lymphocytes that are

abundant in mucosal-associated lymphoid tissue and usually prevent inflammatory reactions against commensal intestinal microbes. The evidence supporting this hypothesis comes from murine biomodels in which the lack of these cells leads to inflammatory bowel disease4. Certain pressure from diners may be mandatory for the preservation of mucosal barrier function. As mentioned above, there are large differences in the composition of the intestinal microbiota between patients with Crohns disease and non-ill subjects, with a low diversity of the microbiome in those who suffer from the disease1–3. Last but not least, the repercussion of these findings should extend to other autoimmune diseases such as diabetes and celiac disease.

Conclusions: Both the IgG serum and IgA salivary responses against S. cerevisiae, D-mannose and D-glucose behaved similarly among the three antigens. Serum specific IgG responses were higher in Crohn's disease, while salivary IgA responses showed an opposite pattern. These antibodies against D-mannose and D-glucose were pioneering described.

REFERENCES

1. Buttó LF, Schaubeck M, Haller D. Mechanisms of microbe-host-interaction in Crohn's disease: Dysbiosis vs. pathobiont selection. Frontiers in Immunology. 2015;6.
2. Buttó LF, Haller D. Dysbiosis in Crohn's disease-Joint action of stochastic injuries and focal inflammation in the gut. Gut Microbes. 2017;8(1):53-8.
3. Pascal V, Pozuelo M, Borruel N, Casellas F, Campos D, Santiago A, et al. A microbial signature for Crohn's disease. Gut. 2017:gutjnl-2016-313235.
4. Wallace KL, Zheng L-B, Kanazawa Y, Shih DQ. Immunopathology of inflammatory bowel disease. World J Gastroenterol. 2014;20(1):6-21.
5. Walker LJ, Aldhous MC, Drummond HE, Smith BR, Nimmo ER, Arnott ID, Satsangi J (2004). Anti-Saccharomyces cerevisiae antibodies (ASCA) in Crohn's disease are associated with disease severity but not NOD2/CARD15 mutations. Clin. Exp. Immunol. 135 (3): 490–96. doi:10.1111/j.1365-2249.2003.02392.
6. Main J , McKenzie H, Yeaman GR, et al. Antibody to Saccharomyces cerevisiae (bakers' yeast) in Crohn's disease. BMJ 1988;297:1105–6. [PMC free article] [PubMed]
7. Sendid B , Colombel JF, Jacquinot PM, et al. Specific antibody response to oligomannosidic epitopes in Crohn's disease. Clin Diagn Lab Immunol 1996;3:219–26.
8. Quinton JF, Sendid B, Reumaux D, et al. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut 1998;42:788–91.
9. Peeters M , Joossens S, Vermeire S, et al. Diagnostic value of anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. Am J Gastroenterol2001;96:730–4.
10. Forcione, DG Rosen, MJ Kisiel, JB Sands BE. Anti-Saccharomyces cerevisiae antibody (ASCA) positivity is associated with increased risk for early surgery in Crohn's disease. Gut 2004;53:1117–1122. doi: 10.1136/gut.2003.030734.

11. Thomsen H, Rose SL, Magno P, Guzman A, Aponte N, Torres EA. Anti-saccharomyces cerevisiae antibodies (ASCA) are present in the saliva of Crohn's disease patients and are useful as an alternative to serum antibodies for diagnosis of Crohn's disease. The American journal of gastroenterology. 2000;95(9):2503.
12. Carey FA, Giuliano RM. Química orgánica. 9 ed. Mexico: McGraw Hill Mexico; 2014.

Effect of Muscarine and Atropine on production of myeloperoxidase by neutrophils in lamina propia of small intestine

Mónica Miguel Santamaría Chavez, Xochitl Abril Rebollar Ruíz, Aldo Arturo Resendiz Albor, Ivonne Maciel Arciniega Martinez, Rafael Campos Rodriguez, Luz María Cárdenas Jaramillo

Instituto Politécnico Nacional, Mexico City, Mexico
yaestabaese@gmail.com; xarebollarr@gmail.com; alrealdo@yahoo.com.mx; ivonne.arciniega.77@gmail.com; citlicampos@gmail.com; luzma9697@gmail.com

Keywords: Atropine, Mice, Muscarine, Neutrophils, Myeloperoxidase (MPO)

The colinergic system it is know as a modulator of the intestinal inmune system, recently have used different drugs to modulat the system and study several aspects of how does it works and how the inhibition or stimulation of muscarine receptor or nicotine receptor can affect different functions of immune system. In this work we will focus on the impact of these two drugs on the production of myeloperoxidase by neutrophils. Three parameters were measured in histologic samples of small intestine: mucus, number and size of goblet cells and number of leucocytes per vili. The mucus layer in the small intestine treated with muscarine is augmented while the group treated with atropine showed no significant difference between the control group. It was also measured the total area of representative goblet cells and obtained the mean value to know if they indeed secreted more mucus, and the groups treated with muscarine where in fact larger than those trated with atropine. On the other hand, there are more goblet cells per area in the group treated with muscarine. Finally the group treated with muscarine show that the number of leucocytes per area of the villi were decreased while in the atropine group the number was slightly augmented. With these results we can make some conclusions and predictions, while atropine shows a protective role in the small intestine, reducing inflammation parameters, muscarine appears to have a proinflammatory effect increasing the production of mucus and the number of leucocytes in the lamina propia. About the mieloperoxidase we can predict that if the number of leucocytes is increased there would be more neutrophils producing more myeloperoxidase.

MIF favors pregnancy-associated periodontitis and oral inflammation in experimental murine model

Betsaida Ortiz-Sanchez, Imelda Juarez Avelar, Miriam Rodriguez-Sosa

Unidad de Investigación en Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla de Baz, Mexico
ortiz.betsy@gmail.com; imelda_juarez@yahoo.com; rodriguezm@unam.mx

Keywords: Periodontal Diseases, Periodontitis, Pregnancy, MMPs, MIF, pregnancy and periodontal disease

Introduction: Periodontitis is a chronic inflammatory disease caused by oral pathogens that affects dental support tissues (periodontium), producing bleeding and gradual bone loss. It affects over 23% women in reproductive age and increases up to 56% in pregnant women. Pregnancy modifies clinical, cytological and microbiological aspects, due to sexual hormones that can alter tissue responses to the oral microbe biofilm presence. Relationship between immunological mediators, like cytokines, expressed in pregnant women and development of periodontitis is not well stablished. Higher levels IL-6 and IL-10 could enhance periodontitis progression. Macrophage migration Inhibitory factor (MIF) is an innate immunity regulator; it favors the toll-like receptor 4 expression and macrophage activation. High levels of MIF have been reported in periodontal tissues, as in crevicular fluid in periodontitis. Moreover, MIF favors osteoclastogenesis and overexpression of metalloproteinases (MMPs) in periodontal disease. These observations suggest that MIF plays a key role in development of periodontal disease, however, the pregnancy-periodontitis-MIF relationship still remain incompletely understood. In this study we evaluated the effect that MIF has on pregnancy-associated periodontitis (PAP) in a murine model.

Methodology: Twenty-five adult (8 weeks old) female BALB/c WT or MIF-knockout (Mif-/-) mice were assigned to groups: PAP, experimental periodontitis (EP), pregnant (P), sham (Sh), and healthy control (CTL). On PAP and EP groups, a 6-0 nylon suture (Ethicon) ligature was placed around maxillary 2nd left molar 2 weeks before mating. The controls P, Sh and CTL did not have ligature. Clinical changes were registered every week. Serum samples from all groups were collected every week, serum cytokines IL-4, IL-6, TNF-α and IFN-γ were assessed by ELISA. Mice were euthanized under CO2/O2 excess atmosphere at 4 weeks after birth, and maxillaries were removed for morphometrically analysis. MMP-2 and MMP-13 were immunohistochemically determined. Data were analyzed statistically by ANOVA and Dunn post-test.

Results: We observed severe histological alterations in the integrity of the junction epithelium in WT groups, and increased insertion deepness in PAP and PE in comparison with CTL mice (Fig 1). In contrast PAP and PE Mif-/- mice developed reduced histological alterations associated to lower insertion deepness (Fig 2). The EP groups

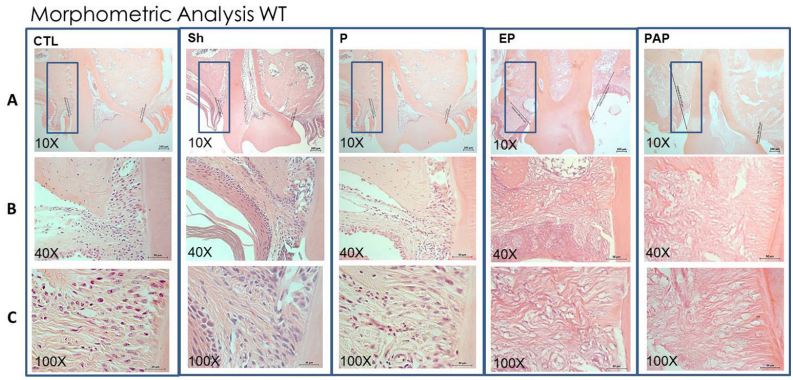


Figure 1: Representative images of periodontal tissues from WT mice. Morphometric analyses were performed in H&E stain representative sections. Panel A, images were measured with ZEN 2 Blue edition (Carl Zeiss Microscopy GmbH) drawing a line between cementoenamel junction and alveolar crest. Data were recorded and analyzed, representative area was enclosed by a blue line. Panel B, representative area enclosed at 40X . Panel c, representative area enclosed al 100X. Healthy control (CTL), sham (Sh), periodontitis (EP), experimental pregnant (P), pregnancy-associated periodontitis (PAP).

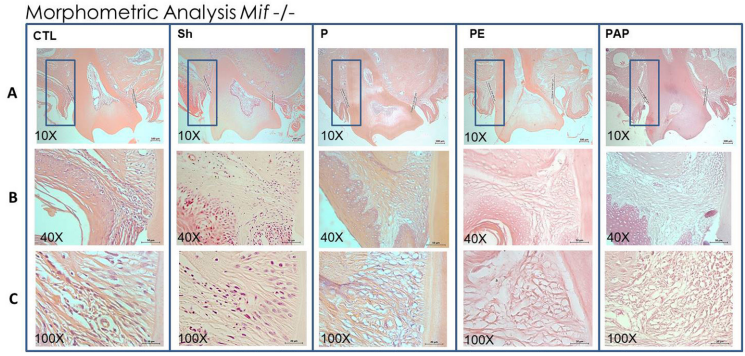


Figure 2: Representative images of periodontal tissues f rom Mif -/- mice. Morphometric analyses were performed in H&E stain representative sections. Panel A, images were measured with ZEN 2 Blue edition (Carl Zeiss Microscopy GmbH) drawing a line between cementenamel junction and alveolar crest. Data were recorded and analyzed, representative area was enclosed by a blue line. Panel B, represen tative area enclosed at 40X . Panel c, representative area enclosed al 100X. Healthy control (CTL), sham (Sh), periodontitis (EP), experimental pregnant (P), pregnancy-associated periodontitis (PAP).

from WT as Mif-/- mice shown higher levels of IL-6 and IFN-γ compared with CTL, Sh and P groups, however EP WT groups showed higher levels of IL-6 compared to EP Mif-/-. No differences were observed in IL-10 and TNF-α expression between WT and Mif-/- groups. We found high expression of MMP-2 and MMP-13 in EP, and higher expression of MMP-2 in PAP WT groups. All groups of Mif-/- showed a lower expression of both MMP's associated to lower periodontitis pathology.

Conclusion: These results suggest that MIF favored periodontal tissue destruction associated with overexpression of MMP-2 and MMP-13, and higher levels of IFN-γ and IL-6 in a murine model of experimental periodontitis. Importantly, pregnancy seems to up-regulate MMP-2 expression promoting the periodontitis pathology. Future studies aim to elucidate the sex hormones role mediating MIF-periodontitis exacerbation.

The effects of an Enriched Environment on the interleukin 18 expression levels in a murine model of colitis

Victor Emmanuel Osio-Becerro, Tomás Villaseñor, Leonor Pérez-Martínez, Gustavo Pedraza-Alva

Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
viktor-osio-6847_@hotmail.com; tomas@ibt.unam.mx; leonor@ibt.unam.mx; gustavo@ibt.unam.mx

Keywords: Colitis, Goblet Cells, Interleukin-18, Inflammatory bowel disease (IBD), Enriched enviroment

The Enriched Environment paradigm (EE) has been studied for many years due to its beneficial effects on the central nervous system and different pathologies. The EE, is defined as housing conditions which promote sensory, visual, cognitive, motor and social stimulation (Nithianantharajah et al., 2006). It has been shown that the EE exerts beneficial effects on the brain at the cellular and functional levels in healthy individuals, promoting neurogenesis and synaptogenesis in the hippocampus through increasing the expression of different neurotrophins such as Brain-Derived Neurotrophic Factor; additionally, the EE prevent memory loss associated with aging (Bruel-Jungerman et al., 2005; Bennett et al., 2006; Kempermann et al., 1997; Rossi et al., 2006). The influence of EE in murine models of different neuropathologies has also been studied, including Down syndrome, Parkinson's disease and Alzheimer's disease, in all cases exposure to an EE ameliorates the symptoms and pathology of each disease (Jankowsky et al., 2005 ; Jungling et al., 2017; Lazarov et al., 2005; Spires et al., 2004;van Dellen et al., 2000). The EE effects are not limited to the nervous system, it also affects the immune system improving chemotaxis, phagocytosis and attenuating the inflammatory response by lipopolysaccharide (Arranz et al., 2010). The EE also prevents or attenuates the development of diseases of the periphery such as obesity and cancer (Cao et al., 2010;2011; Díaz de León et al., in preparation). These pathologies have a common component, an inflammatory process. Inflammatory Bowel disease groups two main pathologies, Crohn's Disease and Ulcerative Colitis, although, these diseases have a wide global distribution, little is known about the factors that lead to their susceptibility. The influence of environmental conditions, genetic elements, immunological factors and the microbiota has been postulated as risk factors to develop these pathologies (Anderson et al., 2011; Akolkar et al., 1997; Frolkis et al., 2013; Kostic et al., 2014). Weight loss, abdominal pain, rectal bleeding, diarrhea and the development of an exacerbated immune response in the gastrointestinal mucosa, increased expression of inflammatory cytokines like IL-1β, IL 6, TNF and IL-18, characterize these disorders

(Nowarsky et al., 2015). Although the role of the various components of the immune system in the development of IBD has been investigated, recently IL-18 is the focus of attention. IL-18 performs a double role in the homeostasis of the intestinal mucosa, IL-18, is necessary for the synthesis of antimicrobial peptides in the intestine, which together with the mucus layer produced by the goblet cells forms a barrier which delimits the interaction of the microbiota with the immune cells present in the lamina propria (Levy et al., 2015). In contrast, increased amounts of IL-18 can have deleterious effects, reducing the expression of key transcription factors involved in the development and maturation of goblet cells, responsible for producing the mucus that covers the intestinal wall (Nowarsky et al., 2015). Given that the exposure to an EE decrease the inflammatory process associated to obesity (Díaz de León et al., in preparation) and colitis (Villaseñor et al., in preparation); we evaluated the effects of the EE on the levels of IL-18 in a murine model of experimental colitis. Here we show that exposure to an EE prevents the raise of IL-18 levels in the colon of mice treated with Dextran sodium sulfate or Trinitrobenzenesulfonic acid compared to those found in animals exposed to the same insults but housed in standard conditions. This correlated with the fact that the number of goblet cells in the colon of mice that were subjected to colitis and exposed to EE were higher than the number of globlet cells found in the colon of mice with colitis housed under standard conditions. The molecular mechanism by which exposure to an EE modulates IL-18 levels is currently under investigation.

REFERENCES

Akolkar, P., Gulwani-Akolkar B., Heresbach, D., Lin, X., Fisher, S. Katz, S. and Silver, J. (1997). Differences in risk of Crohn's disease in offspring of mothers and fathers with inflammatory bowel disease. Am J Gastroenterol. 92:2241-2244.

Anderson, C., Boucher, G., Lees, C., Franke, A., D'Amato, M., et al. (2011). Metaanalysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nature Genetics. 43:246-52.

Arranz, L., De Castro, N., Baeza, I., Mate, I., Viveros, M. and De la fuente, M. (2010). Environmental enrichment improves age-related immune system impairment; long-term exposure since adulthood increases life span in mice. Rejuvenation Research. 13: 415-428.

Bennett, J., McRae, P., Levy, L. and Frick, K. (2006). Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. Neurobiology of Learning and Memory. 85: 139-152.

Bruel-Jungerman, E., Laroche, S. and Rampon, C. (2005). New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. European Journal of Neuroscience. 21: 513-521.

Cao, L., Choi, E. Y., Liu, X., Martin, A., Wang, C., Xu, X. and During, M. J. (2011). White to Brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. Cell Metabolism. 14:324-338.

Cao, L., Liu, X., Lin, En-Ju. D., Wang, C., Choi, E. Y., Riban, V., Lin, B. and During, M.J. (2010). Environmental and genetic activation of a brain-adipocyte BDNF/leptin axis causes cancer remission and inhibition. Cell. 142: 52-64.

Díaz de León-Guerrero S, Meza-Soza K, Jiménez-Jacinto V, Vega-Alvarado L, Pedraza-Escalona M, Salazar-León J, Sanchez-Flores A, Pedraza-Alva G and Pérez-Martínez L. (2018) An Enriched Environment restores glucose homeostasis, reduces inflammation and modifies microRNA levels in the hypothalamus of obese mice. In preparation.

Frolkis, A., Dieleman, L., Barkema, Panaccione, R., Ghosh, S., Fedorak, R., Madsen, K. and Kaplan, G. (2013). Environment and the inflammatory bowel diseases. Canadian Journal of Gastroenterology. 27:18-24.

Jankowsky, J., Melnikova, T., Fadale, D., Xu, G., Slunt, H., Gonzales, V., Youkin, L., Youkin, S., Borchelt, D. and Savonenko, A. (2005). Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. Journal of Neuroscience. 25: 5217-5224.

Jungling, A., Reglodi, D., Karadi, Z., Horvath, G., Farkas, J., Gaszner, B. and Tamas, A. (2017). Effects of Postnatal Enriched Enviroment in a Modelo of Parkinson´s Disease in Adult Rats. International Journal of Molecular Science. 18: 406.

Kempermann, G., Kuhn, H. and Gage, F. (1997). More hippocampal neurons in adult mice living in an enriched environment. Nature 386: 493-495.

Kostic, A., Xavier, R. and Gevers, D. (2014). The Microbiome in Inflammatory Bowel Diseases: Current Status and the Future Ahead. Gastroenterology. 146: 1489-1499.

Lazarov, O., Robinson, J., Tang, Y., Hairston, I., Korade-Mirnics, Z., Lee, V., Hersh, L., Sapolsky, R., Mirnics, K. and Sisodia, S. (2005). Environmental enrichment reduces Aβ levels and amyloid deposition in transgenic mice. Cell. 120: 701-713.

Levy, M., Thaiss, C. A., Zeevi, D., Dohnalá, L., Zilberman-Schapira, G., Mahdi, J. A., David, E., Savidor, A., Korem, T., Herzing, Y., Pevsner-Fischer, M., Shapiro, H., Christ, A., Harmelin, A., Halpern, Z., Latz, Eicke., Flavell, R. A., Amit, I., Segal, E. and Elinav, E. (2015). Microbiota-modulated metabolites shape the intestinal microenviroment by regulation NLRP6 inflammasome signaling. Cell. 163:1428-1443.

Nowarsky, R., Jackson, R., Gagliana, N., de Zoete, Marcel. R., Palm, N. W., Bailis, W., Low, J. S., Harman, C. C. D., Graham, M., Elinav, E. and Flavell, R. A. (2015). Epithelial IL-18 equilibrium controls barrier function in colitis. Cell. 163: 1444-1456.

Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., Fabri, M. E., Tessarollo, L., Maffei, L., Berardi, N. and Caleo, Matteo. (2006). Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. European Journal of Neuroscience. 24: 1850-1856.

Spires, T., Grote, H., Varshney, N., Cordery, P., van Dellen, A., Blakemore, C. and Hannan, A. (2004). Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. Journal of Neuroscience. 24: 2270-2276.

van Dellen, A., Blakemore, C., Deacon, R., York, D. and Hannan, A. (2000). Delaying the onset of Huntington's in mice. Nature 404: 721-722.

Villaseñor T, Nava P, Pérez-Martínez L and Pedraza-Alva G. (2018) Exposure to an enriched environment attenuates mouse experimental colitis. In preparation.

Bacillus subtilis a novel probiotic to improve innate immunity and host health parameters

Roberto Ricardo Grau[1], Federico Argañaraz[1], Antonela Marquez[2], Carlos Bauman[1], Oliver Pérez[3], Paola Gauffin Cano[2]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]Centro de Referencia para Lactobacilos, San Miguel de Tucuman, Argentina
[3]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
robertograu@fulbrightmail.org; robertograu.conicet@gmail.com; anto_m88@hotmail.com; carlosbauman@gmail.com; oliverperez@giron.sld.cu; pgauffin@cerela.org.ar

Keywords: Bacillus subtilis, Galectins, microbiome, mucosal immunity, probiotic spores

Bacillus subtilis is a Gram-positive spore-forming bacterium that is recognized as a probiotic organism. Probiotics are live organisms that have a beneficial effect on host health when they are administered or present in adequate quantities. One global health problem of society concern is the actual epidemic of obesity. The aim of this work was determine if the administration of Bacillus subtilis improves the immunologic and metabolic parameters in normal mice as well as in a fat mice model. A dose of 100 µl of B. subtilis DG101 was administrated daily by gavage at a concentration of the probiotic of 1 x 108 CFU/ml. The mice were fed and weighted daily during 7 weeks, and the consumed food, biochemical and immunologic parameters were determined. The levels of leptin were measured by ELISA and cytokines (TNF-α, MCP, IL-6, IL-10) by flow cytometry. Complement and Galectin-1 levels were measured in blood, liver and spleen only in the standard diet (SD) and SD-Bs groups by indirect ELISA. In the case of fat mice, we used 5 weeks male mice strain C57BBL/6, which has a natural trend to fatten. The mice were divided in two conditions, fed with and without spores of B. subtilis. At the same time, each group was divided in mice fed with a standard diet (SD) and fatty diet (FD). At the end of the experimental period, the mice were fasted for 16 hours and slaughtered with ketamine (150 mg/kg of body weight) and xilazine (5 mg/kg of body weight). We collected blood samples of all the animals (n=24) by cardiac puncture. The plasma was obtained by centrifugation (2500 x g, 10 min) and used for biochemical assays. The concentration of triglycerides, total cholesterol and glucose were measured by enzymatic methods using commercial kits. The adiposity index was calculated by the rate (total body grease/total body weight) x 100. After the blood extraction, the organs (liver, epididimal and mesenteric grease) were surgically removed, washed with phosphate saline buffer, weighted and stored at -70ºC until the analysis. The spores of B. subtilis DG101 were obtained by aerobic fermentation as described.

Effect of the administration of B. subtilis DG101 in immunological parameters

We determined in plasma the levels of cytokines (TNF-α, MCP-1, IL-6, IL-10) 45 days after the killing. There weren´t significant alterations, nevertheless the FD (fat diet) shown a tendency to enhance the levels of TNF-α and MCP-1, at the same time that reduce the levels of IL-6 and IL-10, demonstrating a pro-inflammatory effect compared to the SD. The administration of B. subtilis DG101 restore the levels of pro-inflammatory cytokines (TNF-α and MCP-1), whereas during the administration time it was not observed a change in the level of the anti-inflammatory cytokine IL-10 affected by the FD. The galectin-1 levels observed in mice were significantly higher both in liver and spleen on those animals fed with B. subtilis DG101 compared to the animals of the group SD. These evidence points to an enhanced anti-inflammatory response.

Effect of B. subtilis DG 101 in the corporal body weight and adiposity index

After 7 weeks of feeding the mice with a fatty diet (FD), the weight gain of the FD group was 19,4% higher compared with the animals of the control group fed with

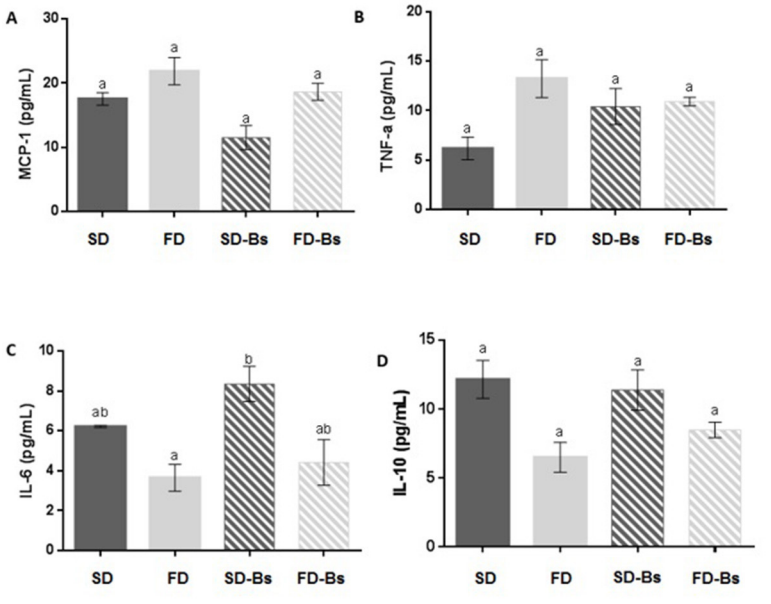


Figure 1: Plasma levels of TNF-α (A), MCP-I (B), IL-6 (C), IL-IO (D), after 45 days of administration of a standard diet (SD), or a fatty diet (FD) with and without supplementation of ***Bacillus subtilis DG101*** (Bs) in a daily dose of 1×10^8 CFU/ml. Each group of data represent the average ± the standard error of 6 mice, different letters indicate significant difference ($p<0.05$) according to statistic t analysis.

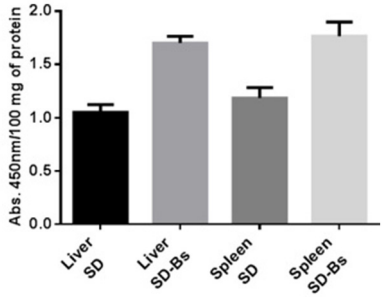


Figure 2: Galectin-I levels in liver and spleen of mice after 45 days of SD feeding with and without supplementation of *Bacillus subtilis DG10I* (Bs) in a daily dose of 1×10^8 CFU/ml. Each group of data represent the average ± the standard error of 6 mice.

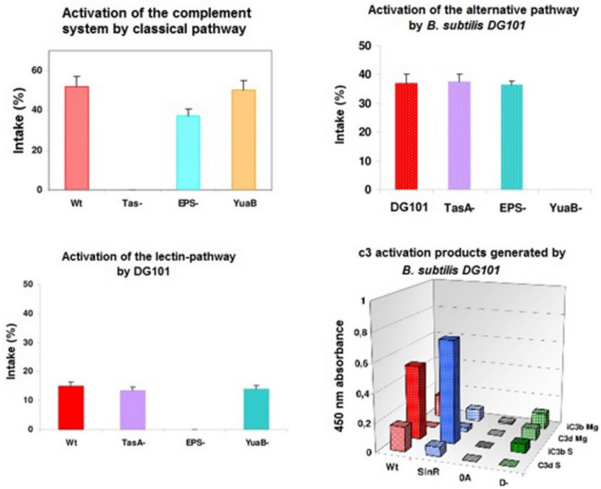


Figure 3: *subtilis DG10I* activates classic (A), alternative (B) and lectin (C) pathways of complement system. The figures A to C show the crucial role of the biofilm matrix components (TasA and BslA proteins and the exopolyshaccaride, EPS) for the specific activation of each complement pathway. Figure D shows the essential roles of the Spo0A and SinR transcription factors for global activation and repression of complement system, respectively.

Table 1: *Weight gain, adiposity index and liver weight.*

Parameter	SD	FD	SD - B. *subtilis* DG101	FD - B. *subtilis* DG101
Weight gain (g)	8.465±0,684	10.108±0.342	6.097±0.551	8.260±0.446
Adiposity index	2.355±0.175	3.573±0.245	1.567±0.131	3.547±0.300
Liver weight (g)	3.450±0.378	4.297±0.249	4.123±0.047	4.145±0.186

Table 2. *Biochemical parameters*

Parameter	SD	FD	SD-B. subtilis DG101	FD-B. subtilis DG101
Triglycerides (g/L)	0.407±0.039	0.483±0.038	0.439±0.122	0.715±0.112
Total cholesterol (g/L)	0.461±0.024	0.711±0.077	0.393±0.042	0.451±0.024
LDL cholesterol (g/L)	0.323±0.013	0.407±0.009	0.238±0.008	0.266±0.041
HDL cholesterol (g/L)	0.262±0.013	0.357±0.053	0.246±0.043	0.456±0.017
Glucose (g/L)	0.620±0.145	2.025±0.433	0.933±0.155	1.112±0.053

standard diet (SD). The animals fed with FD and B. subtilis DG101 shown a decrease of 12,6% of corporal weight compared with the same diet without administration of probiotic.

The adiposity index of the group fed with FD increases significantly 51 % compared with the control group SD. However, the adiposity index of the control group fed with

B. subtilis DG101 decreases significantly 18,2 % compared with the animals of the control group. These results suggest that the administration of the probiotic B. subtilis DG101 favors the decrease of the adiposity index, at least in animals fed with a SD.

The liver weight increases 24% in the FD group compared with the SD group. The administration of B. subtilis DG101 led the liver weight to similar values of the FD group.

There wasn't microbial growth in the plates seeded with supernatant of liver and spleen, so that B. subtilis DG101 don´t affect the permeability of the gut.

Effect of the administration of B. subtilis DG101 in biochemical parameters

The triglycerides values in the FD group were similar to the SD group. Neither exist a significant difference between these groups fed with B. subtilis DG101.

The cholesterol levels in the FD group shown an increase of 54% compared with the animals of de SD group. The mice fed with FD and B. subtilis DG101 return the cholesterol to normal values, similar to the SD group.

The LDL values of the FD group increase significantly about 25% compared to the SD group. The animals fed with FD and B. subtilis DG101 decrease significantly the LDL levels about 34% compared to the FD group.

The HDL levels of the FD group were similar to the SD group. The animals fed with FD and B. subtilis DG101 increase significantly the HDL levels about 74% compared to the SD group. The total cholesterol/HDL rate decrease significantly from 2.15 (FD) to 0.99 (FD-B. subtilis DG101).

The glucose values of the FD group increase twice in regard to the SD group. The administration of B. subtilis DG101 to the FD group decrease the glucose level about 60% compared to the FD group.

Conclusion: The administration of B. subtilis DG101 to mice fed with different types of diet modulates different immunological parameters such as IL-6 and galectin-1, indicating that the bacteria is able to improve the performance of the innate immunity. Biochemical parameters, as body weight, LDL cholesterol, HDL cholesterol and adi-

posity index are also healthy affected by the administration of B. subtilis DG101. The immunologic and metabolic modifications lead to a significant health improvement in the host, which makes B. subtilis DG101 a genuine and powerful probiotic organism.

Lactobacillus reuteri induces gut intraepithelial CD4+CD8 alpha alpha + T cells

Luisa Cervantes-Barragan[1], Jiani N Chai[1], Ma Diarey Tianero[2], Blanda Di Luccia[1], Philip P Ahern[1], Joseph Merriman[1], Victor S Cortez[1], Michael G Caparon[1], Mohamed S. Abou Donia[2], Susan Gilfillan[1], Marina Cella[1], Jeffrey I Gordon[1], Chyi S Hsieh[1], Marco Colonna[1]

[1]School of Medicine, Washington University in St. Louis, St. Louis, United States
[2]Princeton University, Princeton, United States
LCervantes@path.wustl.edu; jchai@wustl.edu; mariadt@princeton.edu; bdiluccia@wustl.edu; Pahern@wustl.edu; jmerriman@wustl.edu; vscortez@wustl.edu; mgcaparon@wustl.edu; donia@princeton.edu; sgilfillan@wustl.edu; mcella@wustl.edu; jgordon@wustl.edu; chsieh@wustl.edu; mcolonna@wustl.edu

Keywords: Lactobacillus reuteri, mucosal immunology, AhR, Intraepithelial lymphocytes, DP IEL

The small intestine contains CD4+CD8 alpha alpha+ double-positive intraepithelial T lymphocytes (DP IELs), which originate from intestinal CD4+ T cells through down-regulation of the transcription factor ThpoK and have regulatory functions. DP IELs are absent in germ-free mice, suggesting that their differentiation depends on microbial factors. We found that DP IEL numbers in mice varied in different vivaria, correlating with the presence of Lactobacillus reuteri. This species induced DP IELs in germ-free mice and conventionally-raised mice lacking these cells. L. reuteri did not shape DP-IEL-TCR repertoire, but generated indole derivatives of tryptophan that activated the aryl-hydrocarbon receptor in CD4+ T cells, allowing ThPOK downregulation and differentiation into DP IELs. Thus, L. reuteri together with a tryptophan-rich diet can reprogram intraepithelial CD4+ T cells into immunoregulatory T cells.

Increased amounts of IgA1 and IgA2 in human colostrum are associated with infections during pregnancy

Erick Sánchez Salguero[1], Leopoldo Santos-Argumedo[1], Hector Romero[1], Kaleb Mondragón[1,2], Rocío Thompson[3], Victor Contreras[3], Julio Alcántara[4], Arturo Cébulo[5]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
[2]Centro Interdisciplinario de Ciencias de la Salud Unidad Milpa Alta, Mexico City, Mexico
[3]Hospital regional 1° de Octubre ISSSTE, Mexico City, Mexico
[4]Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, Mexico City, Mexico
[5]Hospital de la Mujer, Mexico City, Mexico
erick.sanchez@cinvestav.mx; lesantos@cinvestav.mx; hromero@cinvestav.mx; kalebmondragon@me.com; rociothompson@yahoo.com.mx; vicman2yk@hotmail.com; jcalcantara@comunidad.unam.mx; cerbulo@hotmail.com

Keywords: Colostrum, gastrointestinal, Respiratory, mucosal immunity, IgA1, SIgA, Maternal transfer, IgA2

Introduction: Colostrum is produced during the first three days of lactation. It is considered the most important protection-factor for the neonate. It contains a high concentration of cytokines, chemokines, leukocytes and secretory immunoglobulins. Secretory immunoglobulin A (SIgA) is the most abundant antibody in colostrum.

During lactation, maternal SIgA reaches newborn´s respiratory and gastrointestinal tracts. Through a mechanism named "immunological exclusion", it participates in protection at the mucosal epithelia against pathogenic microorganisms, and in the maintenance of homeostasis.

There are two subtypes of IgA in humans: IgA1 and IgA2. The main structural differences between them are their length in the hinge region and their glycosylation profiles. It is known that IgA subclasses have different affinities, depending on the biochemical nature of the antigen. IgA1 has higher affinity to protein antigens and IgA2 against carbohydrates. In addition, their anatomical distribution is well defined, among different compartments of the mucosal epithelia and the secretions.

A higher incidence of infectious diseases correlates with a decrease in the levels of IgA in sera. Epidemiological studies have evaluated the incidence of infectious diseases with

the amount of total IgA in colostrum. Such studies did not find differences between healthy women and women with infectious episodes during pregnancy. Most of these works have been limited to measurements of total IgA levels, without differentiating between IgA1 and IgA2. In Mexico, there are no studies that have quantified immunoglobulin isotypes in the colostrum from healthy women.

Aim: To determine the association between the incidence of infectious episodes during pregnancy with the amount of IgA1 and IgA2 in colostrum.

Results: We quantified immunoglobulins levels in colostrum, with an emphasis in IgA1 and IgA2, from seven hundred healthy women from three Hospitals in Mexico City, using quantitative ELISA technique. The results showed that IgA2 is the most abundant subtype in colostrum. It was observed that more than 90% of total IgA is found as SIgA, which was probably transported by transcytosis; while the remaining 10% was transported by other mechanisms.

Our results showed that, in general, IgA1 is increased and IgA2 decreased in colostrum from women who presented infectious episodes during pregnancy. A correlation was found between the highest levels of IgA1 in colostrum and the number of infections in the respiratory tract and in the skin. In contrast, the number of infectious episodes in the gastrointestinal tract during pregnancy correlated with increased amounts of IgA2 in colostrum.

These results may explain why, in response against respiratory or gastrointestinal tracts infections, higher numbers of plasma cell precursors IgA1 + ($\alpha4\beta1$ +) or IgA2 + ($\alpha4\beta7$ +), respectively, can migrate to the mammary gland and reside in the periphery of the acini, until lactation.

Conclusions: These results provide evidence to understand the effect of immunizations and infections during pregnancy. With these results, we could speculate about the protection given by the colostrum to the newborn. To our best knowledge, this work represents the first experimental evidence, in which, the effect of infectious episodes in women, during pregnancy, have in the concentration of IgA1 and IgA2 in colostrum.

Intestinal immune response to nematodes in pigs

Marco Antonio Vega-Lopez, Héctor Romero, Alonso Benítez, Alberto Piña, Antonieta Lopez

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
mavega@cinvestav.mx; hromero@cinvestav.mx; mavega1@yahoo.com; mavega1@gmail.com; lopezma@cinvestav.mx

Keywords: Cytokines, Intestinal Mucosa, Nematode Infections, mucosal immunity, Swine models

Introduction: Intestinal parasites represent a health problem in developing countries. Hygiene measures are the more effective way to control such diseases. However, general poverty has diminished the life standards in these countries(1). Prevention through vaccination is hampered by the poor understanding of the mucosal immune response elicited by these infections(2). Trichinella spiralis (T. sp.) is a nematode that infects the pig and occasionally humans after the ingestion of contaminated meat with viable larvae. The infective larvae invades the intestinal epithelium, being a multiintracellular parasite, where mates and after few days release infective larvae able to penetrate to the lamina propria (LP) and reach the blood stream to finally lodge in the host´s muscles where it forms a cyst. This type of infection represents a unique opportunity to evaluate the intestinal and systemic immune response in animal models. Many studies have been carried out in rodents (mice and rats), however these models have important dif-

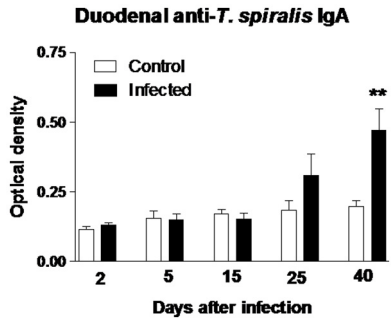


Figure 1: *Anti-Trichinella spiralis* IgA in intestinal content of control (empty bars) and orally infected (filled bars) pigs. Each bar represents the mean + the SEM of five animals at 2, 5, 15, 25 and 40 days alter infection (700 MUkg). - ** p<0.01.

Table 1: Number of intraepithelial lymphocytes in small intestine from *Trichinella spiralis* orally infected pigs (number +/- SEM)

IEL during *T. spiralis infection*			
DAI	Animal group		p<
	CTL	INFECT	
2	71 (16)	109 (25)	0.01
5	0 (0)	0.3 (0.4)	0.05
15	84 (17)	114 (19)	0.05
25	76 (46)	117 (19)	0.05

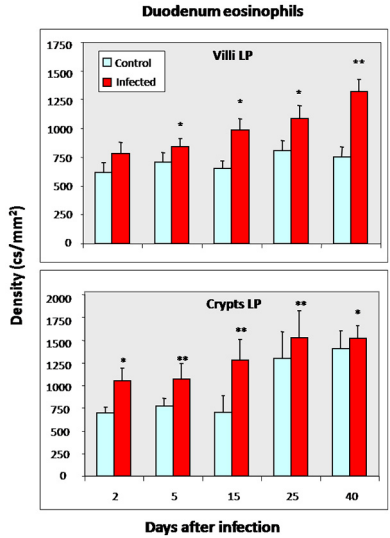


Figure 2: Density (cells/mm^2) of eosinophils in the small intestine of *Trichinella spiralis* orally infected pigs. Each bar represents the mean + SEM of five animals of control (blue bars) and infected (700 ML/kg, red bars) per DAI group. * p<0.05, ** p<0.01.

ferences in the immune response when compared with humans, since rodents are able to get rid of the intestinal parasites within two weeks of the primary infection through

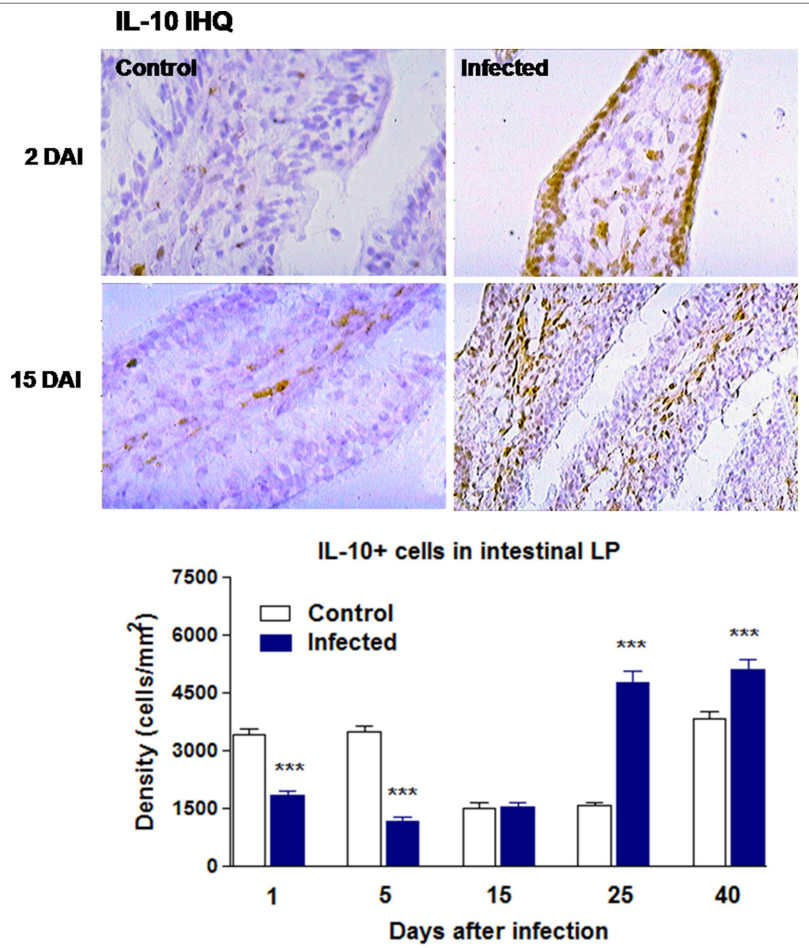


Figure 3: Immunohistochemicalstaining for intestinal LP IL-10+ cells in control (empty bars) and *Trichinella spiralis* orally infected (blue bars) pigs. Each bar represents the mean + SEM of five animals per day alter infection (DAI) group. *** p<0.001.

a vigorous local specific immune reaction mediated by mast cells. In contrast, humans and pigs are unable to mount such response and the infection tends to be chronic(3). In this work, the local and systemic immune response to experimental T. sp. infection was followed in conventional pigs for 40 days, measuring local Ab response by ELISA, intestinal immune cells changes by flow cytometry and immunohistochemistry and

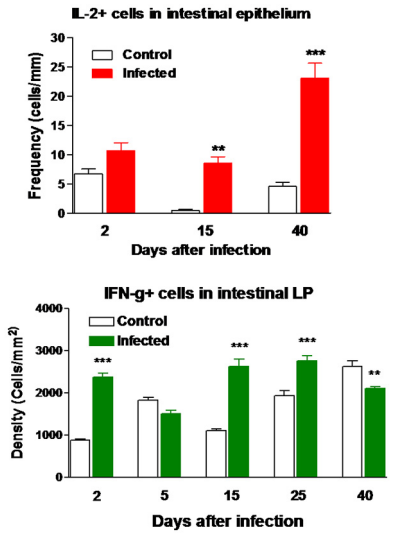


Figure 4: TH1 cytokine profile expression in pig intestinal cells. IL-2 frequency (cells/mm of epithelium) and IFNg density (cells/mm^2 of LP) were counted in control (empty bars) and *Ttichinella spiralis* infected (coloured bars) pigs. Each bar represents the mean and SEM of five animals per DAI group. ** p<0.01,*** p<0.001.

intestinal cytokine production. Our results showed that the parasite was able to partially avoid the local inflammatory response delaying the rise of intestinal Abs and inducing a strong IL-10 response in the intestine.

Experimental Design: Fifty conventionally reared weaned (6 week-old) pigs (Yorkshire x Landrace) from a farm free of enzootic diseases and with no vaccination protocols running were used. The animals were randomly divided in two groups: Control and orally infected with 700 T.sp. infective muscle larvae/kg. Five animals from each group were euthanized with an IV overdose of sodium penthobarbital (Anestesal/Smith Kline Beecham, USA) at 2, 5, 10, 25 and 40 days after infection (DAI). Diaphragm tissue from all animals was collected and checked up for infection.

Results and Discussion: Nematode intestinal infections are prevalent in developing countries. Early treatment is difficult because infection may be unapparent during the first days when the establishment of the parasite and the spread of infection to other organs may be critical. Trichnella spiralis represents a good model to study the local and systemic immune response elicited during infection. Instead using traditional rodent models, we decided to use the pig as being the zoonotic source for humans. Like humans, the pig develops an intestinal infection where the parasite multiplies with no apparent local reaction to eliminate the pathogen(4). Our results showed a delayed IgA response in intestinal content (figure 1), suggesting a strong immune modulation at the inductive sites (e.g. Peyer´s patches) of the host. It was also shown that LP lymphocytes, MHC-II (i.e. macrophages and dendritic cells) and mast cells numbers did not increased in infected animals (data not shown) during the whole experiment, suggesting a strong downregulation of the inflammatory reaction. IELs and eosinophils were the only cells showing significant increases (table 1, figure 2) during infection. IEL are sentinel cells, which usually release TH1 cytokines after activation(5). Eosinophils showed a strong infiltration in intestinal LP as early as 2 DAI (figure 2), first arriving at crypts and later into the villi, their role in parasite infections is still controversial. This is an interesting result since it is known that T. sp. produces high amounts of excretory and secretory antigens which may have immunomodulatory activities(6). Finally, intestinal cells from infected animals did produce cytokines, firstly at the epithelium (figure 3) layer and continuing to the LP at later stages. As early as 2 DAI the IFNg production was significantly increased and its production continued up to day 25 to finally drop below control levels at 40 DAI (figure 4), whilst IL-2 positive cells were higher at the epithelium layer throughout the whole experiment suggesting an early protective TH1 activation (IFNg and IL-2), followed by an increase in IL-10, probably maintaining the infection at the intestine.

These results may help to explain the persistent intestinal (and systemic) infection in the porcine host. Studies on the molecules involved in the local immune modulation may improve our understanding on how to prevent nematode infections at mucosal sites.

REFERENCES

1. J Clin Invest. 2008; 118(4): 1311–1321. doi:10.1172/JCI34261.
2. Immunol Rev. 2011 239(1): 237–270. doi:10.1111/j.1600-065X.2010.00976.x.
3. Vet Parasitol. 2009 159(3-4): 245–248. doi:10.1016/j.vetpar.2008.10.051.
4. Infection, genetics and evolution (2009) 9(4):606-16, DOI:10.1016/j.meegid.2009.03.003.
5. Trends Immunol. 2017: S1471-4906(17)30213-2. doi:10.1016/j.it.2017.11.003.
6. Immunol Res. 2015 61(3):312-25. doi:10.1007/s12026-015-8626-4.

Exposure to all-trans retinoic acid (ATRA) during parenteral immunization increases mucosal immunity in an antigen-specific manner

Bruno Raposo, Michael Reinhart, Rodrigo Gonzalez, Ziqi Chen, Ulrich H. von Andrian

Harvard Medical School, Boston, United States
bruno_raposo@hms.harvard.edu; michael_reinhart@hms.harvard.edu; Rodrigo_Gonzalez@hms.harvard.edu; chen_ziqi@g.harvard.edu; uva@hms.harvard.edu

Keywords: Diarrhea, Intestine, Small, Mucosa, ATRA, gut homing

In developing countries, diarrheal diseases caused by a multitude of enteric pathogens constitute a major cause of death in children <5 years of age. Despite the increased sanitary and socio-economic efforts to reduce child mortality associated with diarrheal diseases, the development of new and more efficient vaccine approaches against enteric pathogens has not seen substantial progress. Parenteral vaccine formulations establish efficient peripheral immune surveillance. However, they generate a very limited protection at mucosal surfaces, such as the gut. Reversely, oral vaccines constitute a good way to induce intestinal mucosa protection. Nevertheless, oral formulations present several limitations, and have been shown to be drastically less efficient in developing countries in comparison to developed countries.

All-trans retinoic acid (ATRA) has been shown to be essential and sufficient in up-regulating gut-homing receptors (α4β7 integrin and chemokine receptor CCR9) in activated T and B cells, both in vivo and in vitro. At steady state, intestinal dendritic cells (DC) are responsible for the restricted synthesis of ATRA in intestinal lymphoid organs (i.e. Peyer's patches and mesenteric lymph node DCs). DCs from other lymphoid compartments, such as the spleen, fail to express the enzymatic machinery required to convert dietary vitamin A to its metabolic active form, ATRA. Nevertheless, in vivo exposure of skin-draining lymph nodes to ATRA during parenteral immunization was shown to result in partial protection from an oral Salmonella challenge. Given these premises, we set to characterize the humoral response elicited by a parenteral immunization upon exposure to ATRA. We assessed antigen-specific antibody responses in the serum and, most importantly, at the intestinal mucosa.

Exposure to ATRA during subcutaneous immunization resulted in a significant increase of serological antigen-specific IgG, similar to that of a standard immunization without ATRA exposure. Interestingly, antigen-specific IgA in the serum was signifi-

cantly elevated in animals exposed to ATRA. At the intestinal mucosa, the detection of antigen-specific IgA was restricted to mice exposed to ATRA. Although we could detect mucosal antigen-specific IgG titers, these did not correlate with ATRA exposure. Moreover, oral challenge with the immunized antigen resulted in a significant increase of intestinal antigen-specific IgA in mice previously exposed to ATRA. These antibody titers remained elevated for a period of 150 days after a single immunization at day 0. This observation suggests that, opposite to parenteral vaccinations, ATRA exposure during immunization is able to establish long-lived plasma cells/memory B cells at the intestinal mucosa. Moreover, these cells are able to respond to an external stimulus, in an antigen-specific manner. The presence of antigen-specific IgA+ cells in the intestinal lamina propria was visually confirmed by two-photon and confocal microscopy.
We have also evaluated the effect of a parenteral boost 5 weeks after the initial immunization. Here, immunized mice were exposed or left unexposed to parenteral ATRA at each of the time points – day 0 and day 35. In this setting, both groups of mice developed a measurable antigen-specific IgA response at the intestinal mucosa. Nevertheless, the titers in ATRA exposed animals were on average 100 fold higher than those observed in immunized control mice. Preliminary data looking at intestinal homing of CD4+ and CD8+ T cells indicates that the mucosal imprinting mediated by ATRA is not restricted to B cells, but also includes antigen-specific T cells activated in the draining lymph nodes.

Looking at draining lymph nodes one week after immunization, we observe an increased frequency of IgA+ cells in mice exposed to ATRA, relatively to control immunized mice. Approximately 50% of these cells express high levels of α4β7, strongly suggesting a mucosal imprinting. Because we can detect antigen-specific IgA responses at the intestinal mucosa after ATRA-exposed immunization, we assessed whether this response was dependent of T cell help. Hence, we immunized MHC-II-/- mice and 2D2 TCR transgenic mice (CD4+ T cells specific for MOG protein) with ovalbumin and exposed them to ATRA. In neither of the mouse strains were we able to detect mucosal ovalbumin-specific IgA, although serological titers of total IgA were normal in these strains when compared to wild-type animals. These observations allow us to conclude that the generation of mucosal antigen-specific IgA antibodies mediated by parenteral ATRA requires antigen presentation to CD4+ T cells, followed by cognate T and B cell interactions. To our surprise, IgA+ cells in draining lymph nodes were positioned outside of the B cell follicles and germinal centers. We are presently characterizing this unusual positioning of T cell-dependent B cells within the lymph nodes. Whether these IgA+ cells express a different set of chemokine receptors, and/or whether they interact with distinct T helper cells is still unknown.

Because α4β7 expression on lymphocytes has also been shown to mediate protection at the vaginal mucosa, we extended our observations to vaginal humoral response elicited after immunization with ATRA exposure. Similar to what we observed at the intestinal mucosa, mice exposed to ATRA also generated vaginal antigen-specific IgA, in contrast to control immunized mice. The titers were substantially increased when the immunization and ATRA exposure were performed through the intra-nasal route, compared to sub-cutaneous route. This observation is akin with oral immunizations, where mucosal antigen exposure is able to elicit a strong mucosa-associated immune response. Interestingly, and regardless of the immunization route, only animals generating an intestinal antigen-specific IgA response showed vaginal titers of the same specificity. Vaginal titers were, however, of a lower order of magnitude.

Together, our results show that addition of ATRA to a subcutaneous immunization has the potential of increasing immune surveillance at mucosal surfaces. At this stage, we still lack solid proof that generation of a strong antigen-specific IgA response at the mucosal surface elicited by ATRA-immunizations is beneficial in a scenario of mucosal infection. Different intestinal pathogens and sexually transmitted pathogens have distinct ways of infecting the host. Hence, whereas an increased humoral response may reduce the infection of certain pathogens, it is possible that in regard to other pathogens it may facilitate their interaction with the host and therefore aggravate the disease. Therefore, all mucosal vaccine approaches mediated by ATRA exposure should be taken into caution.

Characterization of microbiota and probiotics from HIV-positive and negative elderly patients

Mariana Del Rocio Ruiz Briseño, Monserrat Alvarez Zavala, Sarah Ratkovich Gonzalez, Judith Carolina De Arcos Jimenez, Maria Elizabeth Preciado Ortiz, Karina Sanchez Reyes, Moises Ramos Solano, Jaime Andrade Villanueva, Luz A Gonzalez Hernandez

University of Guadalajara, Guadalajara Mexico
lilieth@live.com.m , monse_belan@hotmail.com, sarah.qfb@gmail.com, judcath@gmail.com, elizabeth.nuta@gmail.com, karina_reyesqfb@hotmail.com, biolog.moises@gmail.com, drjandradev@gmail.com, luceroga08@gmail.com

Keywords: HIV Infections, Probiotics, qPCR, elderly patients, microbiota and immunity

Background: Gut microbiota has a significant role in the maintenance of metabolic, physiological and immunological homeostasis of the intestine. HIV infection is characterized by intestinal dysbiosis augmented by diverse external factors such as life style, diet and ageing. The introduction of antiretroviral therapy (ART) has enhanced survival among people living with HIV infection, their life expectancy is close that to HIV- population. Therefore, in recent years, increased rates of comorbid illnesses have been observed in HIV-positive populations, especially in elderly patients. These comorbidities are exacerbated by the dysbiotic state; these patients are characterized by a lower alpha and beta diversity, with a decrease in anti-inflammatory phylum (Firmicutes), and an increase in pro-inflammatory phylum (Bacteroidetes). This process is accompanied with a local and systemic low grade chronic inflammation and affects the gut immune system of HIV+ patients, due to the alteration in the production of its bacterial metabolites such as short chain fatty acids (SCFAs), which help maintain intestinal homeostasis. The alteration in gut microbiota and their metabolites favor the low grade chronic inflammation state and the presence of comorbid illnesses, some of them associated to ageing.

Objective: To characterize and compare some microorganism from gut microbiota, including probiotics such as Clostridium leptum, Clostridium coccoides, Lactobacillus and Bifidobacterium by qPCR in elderly HIV+ and HIV- patients.

Methods: Cross-sectional study; 38 stool samples from elderly patients were included (20 HIV+ and 18 HIV- samples). HIV+ patients: over 50 years old with more than two years of stable ART in virologic control (controlled or undetectable HIV infection

was considered if viral load was ≤ 50 copies/mL under treatment), CD4+ absolute count over 200 cells/μL, with stable body weight; HIV- subjects: over 65 years old (HIV+ patients generally present an immunological attrition comparable to the immunological state of a HIV- person 10 years older) and stable body weight. For both groups the non-inclusion criteria were: use of antibiotics, probiotics, chemotherapy of immunotherapy 30 days before sample taken, Body Mass Index (BMI) ≥30, Diabetes Mellitus, cancer, or co-infection with HBV or HCV. The absolute count of CD4+ T cells (cells/μL), count nadir CD4+ cells were evaluated with FACSCalibur System (Beckton Dickinson, BD). The HIV-1 RNA viral load in plasma was measures through ROCHE Amplicor HIV-1 Monitor 1.5 Ultrasensitive PCR technique with COBAS Ampli-Prep/Cobas Taqman. DNA extraction was performed with QIAamp DNA Stool Mini Kit according to manufacturer´s instructions. Microorganism quantification was done using qPCR in CFX96 thermocycler by BIO-RAD. The primers sets amplified Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Lactobacillus, Prevotella-Bacteroides, Bifidobacterium, C. leptum and C. coccoides; 16S rRNA was used as reference gene. The analysis was performed calculating the ΔCq of each microorganism.

Results: In HIV+ group: the age mean was 57 ± 5 years, CD4+ nadir median was 74 (IQR: 39, 220) cells/μL; the absolute count of CD4+ median was 409 (IQR: 279, 744), and HIV-1 RNA viral load mean was 34 ± 94 copies/μL with a mean of 6 ± 6 ± 3.5 years of undetectable with a NNRTI regimen (TFV/FTC/EFV, co-formulation). In HIV- group, the mean of age was 68 ± 5 years. No significant difference was found in the 16S-rRNA quantification between HIV+ and HIV- group. However, at the level of phylum a significant decrease in Firmicutes ($p<0.05$), a significant increase in Bacteroidetes and Proteobacteria ($p<0.05$) was detected in elderly HIV+ patients; as for the phylum Actinobacteria, no significant difference was quantified. In lactic acid producing genera such as Bifidobacterium and Lactobacillus, no statistical difference was found, but in Prevotella genus a significant decrease ($p<0.05$) was observed in elderly HIV+ subjects. In relation to SCFAs producing Clostridium clusters, no statistical difference was detected for C. coccoides (cluster XIVa), but in C. leptum (cluster IV) a significant decrease ($p<0.05$) was found in elderly HIV+ patients. No correlation was found between absolute count of CD4+ and any of the microorganisms measured.

Conclusions: The reduction of cluster IV Clostridium reflected in the decrease of Firmicutes in addition to the increase of Bacteroidetes and Proteobacteria phylum, shows a dysbiosis process in gut microbiota in elderly HIV+ patients in comparison with elderly HIV- subjects. Due to these characteristics of the elderly HIV+ patients: virologic control and absolute count T CD4+ over 200 cells/μL, no relation between immune status and the dysbiotic state was found.

Diet, microbiota, and bacterial translocation markers in elderly population in Western México

Judith Carolina De Arcos Jimenez, Maria Elizabeth Preciado Ortiz, Mariana Del Rocio Ruiz Briseño, Sarah Ratkovich Gonzalez, Karina Sanchez Reyes, Monserrat Alvarez Zavala, Moises Ramos Solano, Luz Alicia Gonzalez Hernandez, Jaime F Andrade Villanueva

Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
judcath@gmail.com; elizabeth.nuta@gmail.com; lilieth@live.com.mx; sarah.qfb@gmail.com; karina_reyesqfb@hotmail.com; monse_belan@hotmail.com; biolog.moises@gmail.com; luceroga08@yahoo.com.mx; drjandradev@gmail.com

Keywords: Aging, Cytokines, Diet, Inflammation, microbiota

Aging is a continuous and progressive process characterized by decreased functionality of organs and systems, such as the immunologic and digestive. During this stage, the gastrointestinal tract alterations compromise the quality of absorption of the diet this factor together with the senescence state, induce an alteration in the composition of the bacterial populations who reside in this site. Gut microbiota plays an important role in the maintenance of metabolic, physiologic and immunologic homeostasis. Some studies in elderly patients have described a dysbiotic state associated with the colonic permeability; bacterial translocation and their products, like LPS. These factors promote an increase of pro-inflammatory cytokines at systemic level, such as IL-1B, IL-6 and TNF alpha which induce a low-grade inflammation state, characteristic of aging, favoring the presence of comorbidities such as cardiovascular disease, insulin resistance, type 2 diabetes mellitus, frailty, osteoporosis, dementia and cancer. However, the observations on changes in microbial populations are contradictory due to the multiple factors involved, so there is no established pattern.

Objectives: To analyze dietetic characteristics of elderly patients from Western Mexico.

Evaluate the microbiota composition in fecal samples of elderly patients.

Quantify cytokines as well as bacterial translocation markers: I-FABP, LPS, hs-CRP and CD14s in serum of elderly patients.

Methodology: To carry out this descriptive study eighteen subjects over 60 years of age from Western Mexico were recruited. First, a survey was applied with the objective to

evaluate the dietetic characteristics. Additionally, the anthropometric variables weight and height, were taken to calculate BMI.

For the determination of microbiota composition, a stool sample was requested to the patients. DNA extraction was carried out with the QIAamp DNA Stool Mini Kit of QIAGEN according to the manufacturer's instructions. Primers for the identification of Bacteroidetes, Firmicutes, Proteobacterias, Actinobacterias, Enterobacteriaceae, Lactobacillus, Prevotella-Bacteroides, Bifidobacterium, E. coli, C. leptum, C. coccoides were used and 16S rRNA was used as a reference. Microorganisms were quantified by real time PCR using the ΔCq of each microorganism and taking as reference the amplification of the universal 16S rRNA gene.

In serum, levels of total cholesterol, glucose as well as cytokines and bacterial translocation markers IFABP, LPS, CD14s and hs-CRP were measured. A panel of thirteen cytokines was measure using the LEGENDplex Human Inflammation Panel Kit for Flow Cytometry; the results were analyzed with the LEGENDplex™ data analysis software. Finally, the bacterial translocation markers I-FABP, LPS, CD14s and hs-CRP were evaluated by ELISA technique according to the instructions of each manufacturer.

Results: For this study, the group was composed by 11 (61%) men and 7 (39%) women with a mean age of 68 + 4 years. 66% of the subjects did not perform physical activity; the average BMI of the population was 25+ 3 kg/m2, which is considered normal weight. In relation to comorbidities, only hypercholesterolemia was observed in 8 subjects (44%). Regarding the characteristics of the diet, 11 participants (61%) had a consumption of <55% carbohydrates, as well as protein intake of <20%. While 10 subjects (56%) had a lipid consumption of >25%, so their diet is within the recommended parameters for the Mexican population.

In regards of the gut microbiota composition in these patients, at the phylum level a decrease in Firmicutes and Actinobacteria in relation to the proportion of Bacteroidetes and Proteobacteria was detected respectively. There was a minor proportion of Lactobacillus and Bifidobacterias in comparison with Clostridium. A positive correlation of Proteobacteria ($r = 0.532$ $p = 0.023$) and Lactobacillus ($r = 0.506$ $p = 0.032$) relative to age was observed. In relation to the amount of SCFAs producing Clostridium species, C. coccoides and C. leptum were detected, being the latter one the most abundant in our study population.

The evaluated cytokines were: Il-1β, IFN-α, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12, IL-17, IL-18, IL-23 and IL-33. Cytokines were detected in a range of 8.23 to

738.18 (pg/mL), being the most abundant MCP-1, IFN-γ, IL-8 and IL-6. Concerning the bacterial translocation biomarkers, the mean and SD of hs-CRP was 2.12+2 ng/mL, IFABP 2.97+0.22 ng/mL, LPS 66.99+26 pg/mL and CD14s 12.16+4ng/mL. Finally, in relation to microbiota and cytokines a negative correlation observed in C. coccoides with IFN-γ (r = -0.710, p 0.001), Enterobacterias with hs-CRP (r = -0.529, p 0.024) and a positive correlation was observed between the consumption of lipids and MCP-1 (r = 0.496 p = 0.036).

Conclusions: Our results demonstrate that elderly population from Western Mexico present healthy dietetic patrons reflected in their bacterial population, which presented higher levels of Bacteroidetes compared to Firmicutes phylum and greater proportion of probiotics, especially some SCFAs producers groups, which have an important role in defining the pro-inflammatory and anti-inflammatory immune response.

Modulatory effect of moderate exercise on some components of intestinal microbiota of BALB/c mice

Giselle Quezada Ortega[1], Marycarmne Godínez-Victoria[1], Maria Elisa Drago-Serrano[2], Judith Del Carmen Pacheco Yepez[1], Rafael Campos Rodríguez[1]

[1]Escuela Superior de Medicina (IPN), Mexico City, Mexico
[2]Universidad Autónoma Metropolitana, Mexico City, Mexico
qogiselle@outlook.com; maric_27@yahoo.com; dragome@yahoo.com; jpachecoy@ipn.mx; citlicampos@gmail.com

Keywords: Lactobacillus, modularity, Gut homeostasis, Moderate exercise, Microbiota dysbiosis

Introduction: Moderate exercise has been proved to modulate intestinal microbiota that in turn, provides health benefits by preventing the potential colonization of pathogenic agents as well by keeping under control inflammatory responses thus contributing on maintain of gut homeostasis. Justification Study of the modulatory role of exercise on intestinal microbiota may provide experimental background for strategies focused for the prevention and/or control of chronic inflammatory diseases associated with sedentarism and/or aging.

Objective: To analyze the effect of moderate exercise on fecal content of lactobacilli and enterobacteria.

Material and Methods: Six weeks-age old male BALB/c mice were allocated in groups of ten members each as following: exercised group underwent moderate treadmill running and a sedentary mice control group. After completing the exercise protocol for 6 months, fecal pellets from both groups were collected in sterile tubes of 2 mL containing 500 µL sterile thioglycolate broth and homogenized to prepare fecal suspensions. Dilutions 10x of fecal homogenates at a final volume of 1 mL in thioglycolate broth were plated in MRS (for lactobacillus) and MacConkey (for enterobacteria) agar plates and incubated under anaerobic or aerobic conditions respectively for 48 h at 37OC. Colonies were counted and expressed as colony forming units (CFU) per mL of sample. Data comparison between groups were analyzed by Student´s t test and significant differences were regarded at $P<0.05$.

Results: By comparison with sedentary control group, moderate exercise group had significant greater aerobic count ($p<0.015$) while no differences were found in lactobacilli CFU ($p=0.103$).

Discussion: Findings suggested that aerobic bacteria are prone to the upmodulatory role of moderate exercise; underlying mechanism is unknown but probably moderate exercise activate stress signals that favored microenvironmental conditions that enhance the growing of aerobic enterobacteria including increased supply of sugars, oxygen, iron and some others.

Conclusion: Future assays may address the impact of moderate exercise on gut homeostasis or dysbiosis by enhancing aerobic enterobacteria growing.

Dysbiosis in the colon and mesenteric lymph nodes is associated with inflammation and bacterial translocation in an experimental rat model of cholestasis

Natali Vega Magaña[1], Miriam Ruth Bueno[1], Luis Felipe Jave Suárez[2], Leonel Garcia[1], Vidal Delgado Rizo[1], Susana Del Toro-Arreola[1], Laura Sánchez Orozco[1], Rosa Cremades[1], José Sergio Zepeda Nuño[1], Maria Guadalupe Flores Miramontes[2]

[1]*University of Guadalajara, Guadalajara, Mexico*
[2]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
natalivega.dcb@gmail.com; ruthmyriamtop@hotmail.com; lfjave@gmail.com; drleonelgb@hotmail.com; vidalrizo@gmail.com; susanadeltoro@hotmail.com; sanchezorozco.cucs.udg@gmail.com; rosacremades@hotmail.com; patologiabucalgdl@gmail.com; nereyda_2404@hotmail.com

Keywords: Bacterial Translocation, Cholestasis, immune response, Dysbiosis, Mesenteric lymph nodes

Introduction: Bacterial translocation in patients with cirrhosis is an important triggering factor for infections and mortality. In a model of bile duct ligation (BDL), the bacterial translocation appears within 24 hours after liver damage. Nonetheless, the interrelation between intestinal microbiome composition and the inflammatory microenvironment in BDL model has not been described.

Objective: To determinate the microbiome composition in colon stools, mesenteric lymph nodes (MLN), and liver; as well as triggering pro and anti-inflammatory cytokines of bacterial translocation permissibility in BDL model.

Methods: The diversity of microbiota in colon stools, MLN, and liver were determined by 16S rRNA pyrosequencing GS-Junior 454. Cytokine expression in MLN was analyzed by qRT-PCR.

Results: Proteobacteria was the predominant phylum, which translocates to MLN and liver in cirrhotic rats. BDL induced a drastic intestinal dysbiosis at 8 and 30 days post-ligation, which was revealed by an increase in the relative abundance of Sarcina, Clostridium, Helicobacter, Turicibacter, and Streptococcus genera. In contrast,

beneficial bacteria, for instance, Lactobacillus, Prevotella, and Ruminococcus importantly decreased in BDL groups. Mesenteric pro-inflammatory (TNF-, IL-1, IL-6, TLR-4) and regulatory (TGF-, Foxp3, and IL-10) molecules at 30 days post-BDL were significantly increased. Conversely, only TGF- and Foxp3 were significantly augmented at 8 days post-BDL.

Conclusions: Dysbiosis in colon and MLN are linked to an imbalance in the immune response; therefore, this might be an important trigger for bacterial translocation in experimental BDL model.

Differences in the intestinal microbiota structure and in the innate immune response in individuals infected with Blastocystis

Miriam Nieves-Ramírez[1,2], Oswaldo Partida-Rodriguez[1,2], Isabelle Laforest-Lapointe[3], Lisa Reynolds[1], Eric Brown[1], Alicia Valadez-Salazar[2], Patricia Moran-Silva[2], Evan Morien[4], Laura Wegener-Parfrey[4], Jin Minglian[5], Jens Walter[5], Marie-Claire Arrieta[3], Cecilia Ximenez-García[2], Brett Finaly[1,6]

[1]Michael Smith Laboratories, Vancouver, Canada
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[3]Department Head Administrative Assistance, Cumming School of Medicine University of Calgary, Calgary, Canada
[4]Faculty of Science, University of British Columbia, Vancouver, Canada
[5]Agricultural, Food & Nutritional Science, Faculty of Agricultural, Life & Environmental Sciences, University of Alberta, Edmonton, Canada
[6]Faculty of Medicine, University of British Columbia Canada, Vancouver, Canada
mirnieves@yahoo.com.mx; oswpartida@yahoo.com.mx; isabelle.laforestlap@ucalgary.ca; lsareynolds@uvic.ca; ericmichbrown@gmail.com; alicia.vs17@gmail.com; patricia_morans@yahoo.com.mx; morien@zoology.ubc.ca; lwparfrey@botany.ubc.ca; mljn@nwpu.edu.cn; jwalter1@ualberta.ca; marie.arrieta@ucalgary.ca; cximenez2005@yahoo.com.mx; bfinlay@msl.ubc.ca

Keywords: Blastocystis Infections, OTUs, Illumina sequencing of 16S rRNA gene, Calprotectine, IgA Fecal

Recent reports suggest that Blastocystis is associated with compositional and diversity shifts of the bacterial gut microbiota but it is unknown whether these occur from ecological interactions or from the effects of a previous intestinal inflammatory condition in the host. In order to identify potential changes associated with the colonization of Blastocystis, the aim of this study was to compare the fecal bacterial microbiome structure by 16S gene Illumina sequencing and different immunoinflamatory indicators, such as fecal calprotectin and IgA (ELISA), and serum citokyne levels (CBA), between asymptomatic adult subjects with or without Blastocystis intestinal infection. The results suggest that Blastocystis establish an important ecological association with members of the intestinal microbiota (Prevotella copri, Prevotella stercorea, Ruminoccoccus bromii, Alistipes putredinis, Bacteroides sp., Bifidobacterium longum, and Oscillospira sp. [DESeq2, Wald Test and False Discovery Rate (FDR) $p<0.05$]. This balanced interaction finally downregulates the inflammatory response, avoiding tissue damage and disease.

Two new in vivo model systems to directly examine human gut microbiota's role in human T cell development and immunity

Baolin Liao, J. Victor Garcia, Angela Wahl

[1]Guangzhou Eighth People's Hospital, Guangzhou, China
[2]University of North Carolina at Chapel Hill, Chapel Hill, United States
baolin_liao@med.unc.edu; victor_garcia@med.unc.edu; awahl@med.unc.edu

Keywords: Immunity, T cells, microbiome, Humanized mouse models, in vivo models, humanized mice

The composition of the gut microbiome is perturbed in patients with inflammatory bowel diseases. Associations between gut microbiota and colorectal cancer, diabetes, and obesity have also been described. However, it is not clear if the altered gut microbiome or particular gut microbiota are the cause or effect of disease. The type of direct experimentation that is needed to address these and other questions of fundamental importance is not possible to perform in humans. Therefore, new in vivo models are needed to address this important gap in our knowledge. Humanized mice have been extensively utilized to study human immune development and diseases. However, to date the gut microbiome of all types of humanized mice is of murine origin and there is very little overlap between the gut microbiome of humans and mice. To study the human gut microbiome's role in human T cell development and immunity during health and disease, we 1) derived and utilized a germ-free immune deficient mouse strain to generate germ-free bone marrow/liver/thymus (BLT) humanized mice, 2) characterized human immune cell reconstitution of germ-free BLT mice, and 3) colonized germ-free mice with human gut microbiota to create BLT mice with a human microbiome (HuM-BLT). A germ-free NOD/SCID/IL2Rγc-/- (NSG) mouse strain was surgically derived by sterile embryo transfer into gnotobiotic recipients. Germ-free BLT mice were generated in a germ-free surgical isolator by transplanting human hematopoietic stem cells into irradiated germ-free NSG mice implanted with autologous human thymus and liver tissue under their kidney capsule. Flow cytometric analysis revealed robust systemic reconstitution of germ-free BLT mice with human immune cells including dendritic cells, macrophages, NK cells, B cells, and T (CD4+ and CD8+) cells. Most importantly, we identified the presence of Treg, TH17, and tissue-resident memory T cells (TRM) in gut of germ-free BLT mice and, as observed in humans, the majority of T cells (53.2%) expressed an effector memory phenotype (CD45RO+CCR7neg). 16S amplicon sequencing of fecal pellets collected from mice demonstrated that the

microbiome of germ-free mice colonized with human gut microbiota recapitulated the human donor inoculum (average Bray-Curtis similarity 63.9) and was distinct from mice colonized with mouse gut microbiota (average Bray-Curtis similarity 1.4). Most importantly, the human gut microbiome was reproducibly maintained over time in all animals (Kruskal-Wallis test) with no statistical difference between HuM-BLT mice analyzed at 14 weeks post-colonization in the taxa present (>1%, Kruskal-Wallis test).

Macrophages and Breast Cancer Interactions: A Dynamical Approach

Ugo Enrique Avila-Ponce de León[1,2], Osbaldo Resendis-Antonio[2,3,4]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]National Institute of Genomic Medicine, Mexico City, Mexico
[3]Red de Apoyo a la Investigación (RAI), UNAM, Ciudad de México, Mexico
[4]Center for Complexity Sciences, National Autonomous University of Mexico, Mexico City, Mexico
uapdl@hotmail.com; oresendis@inmegen.gob.mx;

Keywords: Macrophages, Tumor Microenvironment, breast cancer, S-systems, Systems Immunology, Boolean modelling

In the human body the immune system is the first line of defense when a strange agent can cause any damage in our tissues. Macrophages are cells of the immune system whose presence within the tumor microenvironment correlate with a poor prognosis in breast cancer. The peculiarity of macrophages is that they present a phenotypic differentiation at the moment when the M1 (anti-tumor) interact with the cancer cells, changing their properties to M2 (pro-tumor). The function of the anti-tumor macrophage (M1) is to release in the microenvironment cytokines that affects cancer proliferation and induces apoptosis, instead the pro-tumoral macrophage (M2) that liberates growth factors like epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) to favour cancer progression. Understand the mechanism by which macrophages participate in the promotion of cancer is a fundamental question with a strong impact on therapeutic applications.

In order to evaluate the role that macrophages and its transition from M1 to M2 have in the cancer development, in this paper we reconstruct a network involving signaling molecules between cancer cells and macrophages in M1 and M2 states and evaluate its phenotypes through mathematical approaches. The network was reconstructed based on immunological theory concerning macrophage polarization and the interaction with breast cancer. Having the reconstruction, we used two complementary mathematical approaches to evaluate their phenotype capacities: Boolean and differential equations with Power-law formalism. We considered the tumor microenvironment generated between the macrophages and the mammary tumor that favors or not the tumor progression in both approaches.

Dynamic Boolean analysis of the network let us to conclude the following. We obtained two attractors: one where cancer cells proliferates and the other where cancer is eliminated. In the attractor where cancer proliferates, the pro-tumoral macrophage (M2)

is present.It generates a favorable microenvironment for the progression of cancer due to the release of growth factors and cytokines the inactivates the cytotoxic action of T cells. In the second attractor, where cancer cells do not survive, the anti-tumor macrophages is present, liberating in the microenvironment cytokines that suppresses cancer proliferation and the inactivation of pro-tumor action of M2 and regulatory T cells. Furthermore, our model concludes that of all the possible combinations of initial states, 82% does not allow the proliferation of cancer cells, the resulting 18% allows cancer progression. Our analysis suggests that the immune system is able to eliminate cancer, and the macrophage is important due its ability to regulate the microenvironment toward a pro or anti tumor action.

On the other side, using the differential equations and the power law formalism, we studied the temporal response and dynamical stability around a steady-state condition of the system integrated by M1, M2 and cancer cells. We concluded that one of the scenarios that caused instability occurs when the levels of inhibition of M2 cells to M1 cells was high, this promoted the increase of cancer cell levels. Another case of instability was caused by the cytotoxic action of M1 cells to cancer cells and the inhibition of M2 cells, thus not allowing the proliferation of cancer cells and not permitting the recruitment of regulatory cells by the M2 cells, the levels of M2 cells was reduced by these instability. From the sensitivity analysis, we observed that if we increase in 1% the parameter of inhibition of M1 to M2, then the dynamics of the three population change to induce a higher antitumor than pro-tumor cells. This being said the proliferation of cancer will be diminished and the population of M2 cells will be sufficient to regulate the cytotoxic action of M1 cells, the final outcome will be the elimination of cancer and the resolution of the inflamation.

Overall, we concluded that our mathematical analysis of the interaction between macrophages and mammary tumor cells demonstrate the importance of the role of macrophages in breast tumor progression, both in its role of generating a favorable microenvironment for tumor settlement and in its role of immunosurveillance. They also point out the importance of certain substances found in the tumor microenvironment that induce the polarization of the macrophage.

MiRNAs expression profile at early rheumatoid arthritis and validation of hsa-miR-361-5p as biomarker for early diagnosis

MF Romo-García[1,2], JC Fernández-Ruíz[1,2], Y Bastian[2], M. Zapata-Zuñiga[3], Noe Macias-Segura[2], JD Castillo-Ortiz[4,5], EE Lara-Ramírez[2], AJ Berlanga-Taylor[6], R González-Amaro[1], C Ramos-Remus[4,5], JA Enciso-Moreno[2], Julio Enrique Castañeda Delgado[2]

[1]Facultad de Medicina, Universidad Autónoma de San Luis Potosi, San Luis Potosí, Mexico
[2]Unidad de Investigación Biomédica de Zacatecas (IMSS), Zacatecas, Mexico
[3]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[4]Unidad de Investigación en Enfermedades Crónico Degenerativas, Instituto Nacional de Enfermedades Respiratorias (INER), Guadalajara, Mexico
[5]Universidad Autónoma de Guadalajara, Guadalajara, Mexico
[6]School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom
mromog@cinvestav.mx; julio137.jc@gmail.com; ybastianhe@conacyt.mx; martinzz1@yahoo.com; roca148@hotmail.com; drcastilloortiz@gmail.com; elarar0700@hotmail.com; a.berlanga@imperial.ac.uk; rgonzale@uaslp.mx; r_ramos@prodigy.net.mx; enciso_2000@yahoo.com; julioenrique_castaeda@yahoo.com.mx

Keywords: miRNA, regulation, biomarker, Pathogenesis, CCP, early rheumatoid arthritis, expression profile.

Introduction: Rheumatoid arthritis (RA) is an autoimmune disease characterized by the symmetrical appearance of symptoms in joints and by morning stiffness longer than one hour. The average prevalence of rheumatoid arthritis worldwide is 0.5 to 1.1% [1] affecting three times more women than men [2]. Autoantibodies such as anticitrullinated protein antibodies (CCP) are detectable months to years prior to clinical manifestations [3] and have a significant relationship with the development of RA [4–6]. Such antibodies on RA have a sensitivity of 72% and a specificity of 92% [7,8] ,however, these antibodies are not exclusive to RA.

Several detectable changes occur in preclinical stages in patients with early arthritis (less than 6 months) at translational [9] level, the main regulators of gene expression are the microRNAs (miRNAs). MiRNAs are small non-coding RNA molecules containing about 22 nucleotides that inhibit gene expression through different mechanisms that block mRNA translation [10]. During the last decade it has been described that

miRNAs could contribute to rheumatoid arthritis (RA) physiopathology. The deregulated miRNAs are very likely to be used in early RA stages as biomarkers. The early detection resides on the increase of remission possibilities, in a range of 40% to 60% of patients [11].

Objective: Identify a profile of overexpressed miRNAs by microarray analysis of early rheumatoid arthritis and asses its sensitivity and specificity as diagnostic biomarkers

MATERIALS AND METHODS:

Sample Collection and RNA Extraction

We collected 4 to 5 ml of whole blood in EDTA tubes plus 1ml of RNA later. The subjects were diagnosed according to the selection criteria (ACR Eular 2010) and classified on CTRL (healthy controls), ACCP+ (positives to CCP antiobodies) , ART (early rheumatoid arthritis) and ARE (established rheumatoid arthritis), this classification was developed according to the characteristics of the natural illness history [16] [17]. RNA was extracted from whole blood with Absolutely miRNA, RNA kit (Agilent) following the manufacturer instructions.

Microarray hybridization and data analysis

The procedure was made with 100ng of total RNA following the manufacturer instructions for Agilent Human miRNA Microarray, the microarray content probes for 1205 human miRNAs (cat. Num. G4870A). Arrays information was extracted using the Agilent Scanner and Agilent Feature Extraction Software (v10.7.3.1). Further analyses were performed using GeneSpring GX version 14.5 software (Agilent) with default settings. Differential expression analysis was performed with an ANOVA with Benjamini-Hochberg correction. Probes with P-value < 0.05 were considered for further analysis.

RT-qPCR

RNA was retrotranscribed to cDNA by the use of a stem-loop primer and U6 RT primer as previously described by Chen et al[18][19] with SuperScript™II Reverse Transcriptase kit (Invitrogen, USA) and 150ng of total RNA (50ng/µl). Real-time PCR quantification was performed with SsoFast-Eva green (Bio-Rad) using standard amplification conditions[18]. The miRNAs expression assay was quantified by the 2-ΔΔCq method and normalized to U6 expression. Primer sequences for qPCR were Foward for U6 5'-GCTTCGGCAGCACATATACTAAA-3´, Reverse U6 5'-CGCTTCACGAATTTGCGTGTC-3´, miR-361-5p 5´-ATTATCAGAATCTCCAGGGGTAC-3´ and Universal 5´-ACGAGGAAGAAGACGGAAGAA T-3´.

Statistical Analysis

Differences in miRNA expression detected by microarray were calculated by ANOVA for microarray Data with GeneSpring Software. In order to validate the microarray results differences in miRNA expression measured by RT-qPCR were calculated by Kruskal-Wallis and Dunn´s post-test. To determine the potential of has-miR-361-5p as biomarker of early RA, receiver operating characteristic (ROC) curve was performed to calculate specificity and sensitivity.

Results:

Microarray Analysis Reveal a Specific miRNA Signature for RA

The miRNA expression profile on RA stages was studied using the Agilent Human miRNA Microarray on a group of 5 subjects per group, our study was designed to identify miRNAs expression at different stages of disease simulating a course progression from preclinical to clinical stages. 253 human miRNAs approved the detection criteria and showed a significant ($p < 0.05$) differential expression among all groups (FIGURE 1 A and B).

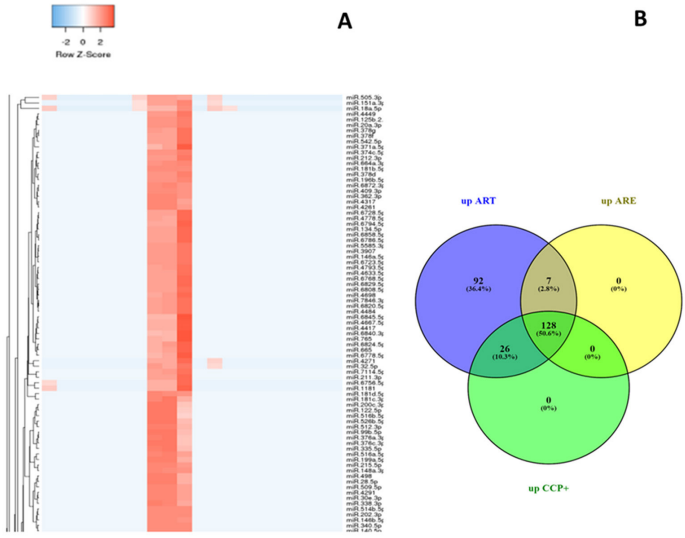


FIGURE 1: Unsupervised hierarchical clustering of globally expressed miRNAs. A) Heat map of 92 miRNAs (p-value <0.05) over expressed on early RA. Top legend (blue to orange) depicts range of expression values. White coloring indicates expression below the detection limit. Color coding corresponds Grey = established rheumatoid arthritis, Dark Red = early rheumatoid arthritis, Blue = positives to CCP and Red = Healthy controls. B) Venn diagram depicting overexpressed miRNAs fingerprint per group compared to controls.

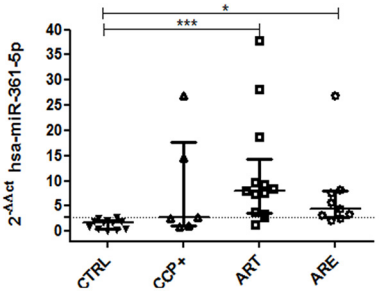
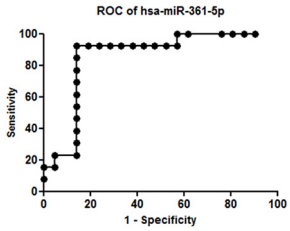


FIGURE 2: Relative expression of hsa-miR-361-5p in whole blood from healthy control subjects (CTRL), patients with established rheumatoid arthritis (ARE), subjects positives to CCP antibodies (CCP+) and patients with early rheumatoid arthritis (ART). hsa-miR-361 -5p relative express ion in whole blood of CTRL (filled triangles), ARE (open circles), CCP+ (open triangles) and ART (open squares), median values analyzed by Kruskal-Wallis test with Dunn's test for multiple comparisons. Dashed line determines the Cut-off point of ROC curves. Error bars are the fir st and third quartiles (*p<0.05 , **p<0.01 and *** P< 0.001).



ROC curves results

miRNA	Specificity	Sensitivity	Cutt-off	A.U.C.	*p* Value
miR-361-5p	85.71	92.3	2.74	0.853	*0.006*

Specificity and sensitivity of mirnas expression determined of comparrison between ART miRNAs expression vs CTRL-CCP+ group

FIGURE 3: ROC curves (representing 1-specificity vs. sensitivity values) for ART patients vs. non RA group (CTRL and CCP+). Curve was calculated using 2 -ΔΔ CT hsa-miR-361-5p values.

Validation of hsa-miR-361-5p Expression by RT-qPCR and Biomarker Capacity

The hsa-miR-361-5p was selected based on the lowest p-value and highest fold change, its expression was measured by RT-qPCR in 12 CTRL, 6 CCP+, 13 RA and 9 CRA and resulted significant overexpressed in ART compared to the other groups, consistent with the microarray data (FIGURE 2). We further proceed to measure its potential as biomarker. ROC curve results pointed that hsa-miR-361-5p presented a specificity and a sensitivity of 92.3 and 85.7 respectively and (AUC>0.9, $P < 0.05$) (FIGURE 3).

Conclusion: We describe a specific transcriptional fingerprint of miRNAs on RA early stages which may have a potential as early biomarkers. This miRNA expression profile identifies several miRNAs that have not previously been associated to RA pathogenesis. In this work, we demonstrate that hsa-miR-361-5p is overexpressed in early RA and have a potential as a biomarker.

REFERENCES

[1] G.J. Tobón, P. Youinou, A. Saraux, The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis, J. Autoimmun. 35 (2010) 10–14. doi:10.1016/j.jaut.2009.12.009.

[2] I. PELAEZ-BALLESTAS, L.H. SANIN, J. MORENO-MONTOYA, J. ALVAREZ-NEMEGYEI, R. BURGOS-VARGAS, M. GARZA-ELIZONDO, J. RODRIGUEZ-AMADO, M.-V. GOYCOCHEA-ROBLES, M. MADARIAGA, J. ZAMUDIO, N. SANTANA, M.H. CARDIEL, Grupo de Estudio Epidemiológico de Enfermedades Músculo Articulares (GEEMA), Epidemiology of the Rheumatic Diseases in Mexico. A Study of 5 Regions Based on the COPCORD Methodology, J. Rheumatol. Suppl. 86 (2011) 3–8. doi:10.3899/jrheum.100951.

[3] J. Kay, K.S. Upchurch, ACR/EULAR 2010 rheumatoid arthritis classification criteria, (n.d.). doi:10.1093/rheumatology/kes279.

[4] J. Laki, E. Lundström, O. Snir, J. Rönnelid, I. Ganji, A.I. Catrina, C. Bengtsson, S. Saevarsdottir, M.C. Wick, L. Alfredsson, L. Klareskog, L. Padyukov, Very high levels of anti-citrullinated protein antibodies are associated with HLA-DRB1*15 non-shared epitope allele in patients with rheumatoid arthritis, Arthritis Rheum. 64 (2012) 2078–2084. doi:10.1002/art.34421.

[5] Y. Okada, K. Kim, B. Han, N.E. Pillai, R.T.-H. Ong, S. Raychaudhuri, Risk for ACPA-positive rheumatoid arthritis is driven by shared HLA amino acid polymorphisms in Asian and European populations, Jian Yin Sang-Cheol Bae Huji Xu. 111281517 (n.d.). doi:10.1093/hmg/ddu387.

[6] L. Padyukov, C. Silva, P. Stolt, L. Alfredsson, L. Klareskog, R. Holmdahl, J. Rönnelid, L. Klareskog, S. Rantapää-Dahlqvist, A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis, Arthritis Rheum. 50 (2004) 3085–3092. doi:10.1002/art.20553.

[7] R. Aggarwal, K. Liao, R. Nair, S. Ringold, K.H. Costenbader, Anti-citrullinated peptide antibody assays and their role in the diagnosis of rheumatoid arthritis., Arthritis Rheum. 61 (2009) 1472–83. doi:10.1002/art.24827.

[8] Z. Szekanecz, L. Soós, Z. Szabó, A. Fekete, A. Kapitány, A. Végvári, S. Sipka, G. Szücs, S. Szántó, G. Lakos, Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis: As Good as it Gets?, Clin. Rev. Allergy Immunol. 34 (2008) 26–31. doi:10.1007/s12016-007-8022-5.

[9] K. Raza, F. Falciani, S.J. Curnow, E.J. Ross, C.-Y. Lee, A.N. Akbar, J.M. Lord, C. Gordon, C.D. Buckley, M. Salmon, Early rheumatoid arthritis is characterized by a distinct and transient synovial fluid cytokine profile of T cell and stromal cell origin., Arthritis Res. Ther. 7 (2005) R784-95. doi:10.1186/ar1733.

[10] J. Mathieu, H. Ruohola-Baker, Regulation of stem cell populations by microRNAs., Adv. Exp. Med. Biol. 786 (2013) 329–51. doi:10.1007/978-94-007-6621-1_18.

[11] M. Schneider, K. Krüger, Rheumatoid arthritis--early diagnosis and disease management., Dtsch. Arztebl. Int. 110 (2013) 477–84. doi:10.3238/arztebl.2013.0477.

[12] D.A. Walsh, D.F. McWilliams, Mechanisms, impact and management of pain in rheumatoid arthritis, Nat. Rev. Rheumatol. 10 (2014) 581–592. doi:10.1038/nrrheum.2014.64.

[13] L. Hunt, P. Emery, Defining populations at risk of rheumatoid arthritis: the first steps to prevention, Nat. Rev. Rheumatol. 10 (2014) 521–530. doi:10.1038/nrrheum.2014.82.

[14] L.H. Yang, S.L. Wang, L.L. Tang, B. Liu, W. Le Ye, L.L. Wang, Z.Y. Wang, M.T. Zhou, B.C. Chen, Universal stem-loop primer method for screening and quantification of microRNA, PLoS One. 9 (2014) 1–13. doi:10.1371/journal.pone.0115293.

[15] P.S. Steeg, L. Kopper, U.P. Thorgeirsson, E. Talmadge, et. al., Stem-loop RT-qPCR for miRNAs, J. Biol. Chem. 7 (2013) 355–62. doi:10.1002/0471142727.mb1510s95.STEM-LOOP.

Immunoinformatic approach to the selection of animal models of hypersensitivity based on the similarity of two molecular targets at genomic level

Orlando Rafael Serrano-Barrera[1,2], Oliver Pérez[3]

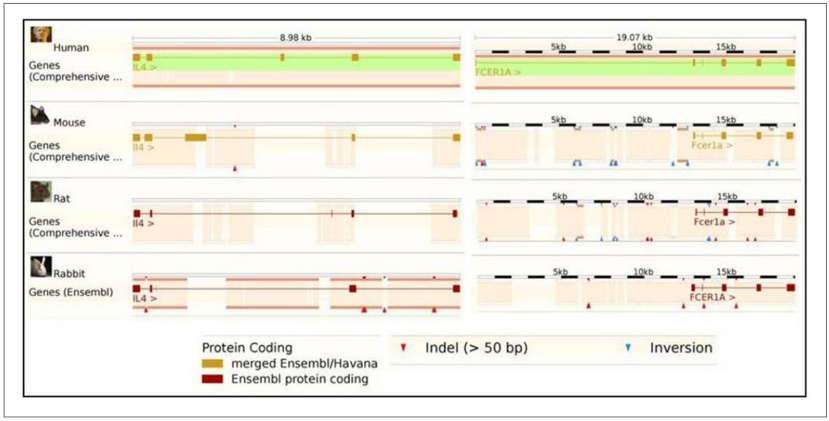
[1]Las Tunas General Teaching Hospital, Las Tunas, Cuba
[2]Medical University of Las Tunas, Las Tunas, Cuba
[3]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
orlandosb@infomed.sld.cu; oliverperez@giron.sld.cu

Keywords: Computational Biology, Hypersensitivity, bioinformatics, IgE, allergy, interleukin 4, laboratory animals

Bioinformatics is applicable to the design of drugs, the simulation of biological effects and in the inter-species comparison of molecules involved in different phenomena and diseases, such as allergies, an important and growing health problem. (1) Omics technologies may be useful to select the best animal model, to model the experiments just after their design and to interpret results. In order to compare two key molecules in allergic conditions, by means of bioinformatic tools, between man and other animal species, interleukin 4 (IL-4) and high affinity Fc receptor of immunoglobulin E were selected as target molecules in four species: man (Homo sapiens), mouse (Mus musculus), rat (Rattus norvegicus) and rabbit (Oryctolagus cuniculus).

Data from genes were retrieved from Gene database (https://www.ncbi.nlm.nih.gov/gene/). Genomic level comparison was carried out by Ensembl (http://www.ensembl.org), (2) while multiple sequence alignments were done with MUSCLE tool (http://www.ebi.ac.uk/Tools/msa/muscle/) and identity matrices were generated by Clustal2.1. UCSC Genome Browser (http://genome.ucsc.edu) was used for the graphical representation of sequence alignments and the occurrence of single nucleotide polymorphisms. (3)

IL-4 is a key regulator of a number of immune functions (4) and is an attractive target for the development of drugs for allergies and other diseases (5). The best similarity was found between composition and location of coding sequences from man and rabbit for IL-4, although a greater number of insertions and deletions was present in the latter (figure 1). Abnormal levels of IL-4 have been reported in a rabbit model of Henoch-Schönlein purpura (6,7), as well as the role of IL-4 in the pathogenesis of lung cavities by tuberculosis both in humans and rabbits (8).

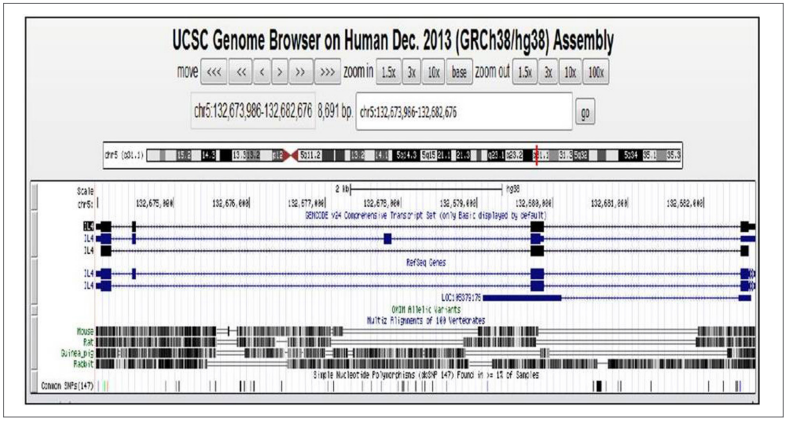


In the case of FcεR1a, the rabbit was the animal model with a lower number of insertions/deletions and no inversions, when compared to human. Details of the sequence and other annotations of FcεR1a in the rabbit and its orthologs, including human, are available in databases such as Gene (https://www.ncbi.nlm.nih.gov/gene/100349846), Ensembl (http://www.ensembl.org/Oryctolagus_cuniculus/Gene/Summary?db=core;g=ENSOCUG00000022947;r=13:33924333-33932430;t=ENSOCUT00000021281) and KEGG (http://www.genome.jp/dbg et-bin/www_bget?ocu:100349846). The genetic basis of rabbit IgE in comparison to the human one has also been assessed. (6)

Multiple sequence alignments for both molecules showed the highest scores of similarities in sequences from rabbit and man: 64,89 % in the case of IL-4 and 71,14 % for FcεR1a.

Single nucleotide polymorphisms (SNPs) predominated in non-coding regions of IL-4 (figure 2), and SNPs were not available for FcεR1a in UCSC Genome Browser. Polymorphisms in IL-4 have been linked to atopic dermatitis, and controversial results have been reported for other conditions, such as asthma and cancer (9–12). SNPs should also be taken into account when assessing drugs: monoclonal antibodies, anti-IL-4 vaccines and anti-sense oligonucleotides (4).

IL-4 and FcεR1a have influences over IgE levels and other immune events, and genome-wide association studies have identified both genomic regions, 1q23 and 5q31, with blood concentrations of the antibody (13,14).



The results reported here support the usefulness of rabbit as a model for human diseases related to allergies, based on the similarities for both species in terms of the two molecules that are considered key in hypersensitivity phenomena. The integration of bioinformatic tools and resources, omics technologies, system biology and other emerging disciplines, will significantly contribute to the comprehension of allergies in particular and all life processes in general.

REFERENCES

1. Romero-Fernandez W, Batista-Castro Z, De Lucca M, Ruano A, García-Barceló M, Rivera-Cervantes M, et al. El 1, 2, 3 de la experimentación con animales de laboratorio. Rev Peru Med Exp Salud Pública Bioética. 2016;33(2):288-99. Available at: http://www.rpmesp.ins.gob.pe/index.php/rpmesp/article/view/2169.
2. Yates A, Akanni W, Amode MR, Barrell D, Billis K, Carvalho-Silva D, et al. Ensembl 2016. Nucleic Acids Res. 2016;44(D1). Available at: http://nar.oxford journals.org/content/44/D1/D710.full.
3. Speir ML, Zweig AS, Rosenbloom KR, Raney BJ, Paten B, Nejad P, et al. The UCSC Genome Browser database: 2016 update. Nucleic Acids Res. 2013;44(D1):D717-25. Available at: http://nar.oxfordjournals.org/content/44/D1/D717.long.
4. Tan HT, Sugita K, Akdis CA. Novel Biologicals for the Treatment of Allergic Diseases and Asthma. Curr Allergy Asthma Rep. 2016, Oct;16(10):70. Available at: http://link.springer.com/article/10.1007/s11882-016-0650-5.
5. Kau AL, Korenblat PE. Anti-Interleukin 4 and 13 for Asthma Treatment in the Era of Endotypes. Curr Opin Allergy Clin Immunol. 2014, Dic;14(6):570-5. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/251591 82/.
6. Gertz EM, Schäffer AA, Agarwala R, Bonnet-Garnier A, Rogel-Gaillard C, Hayes H, et al. Accuracy and coverage assessment of Oryctolagus cuniculus (rabbit) genes encoding immunoglobulins in the whole genome sequence assembly (OryCun2.0) and localization of the IGH locus to chromosome 20. Immunogenetics. 2013, Oct;65(10):749-62. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3780782/.

7. Li Y, Feng X, Huang L, Zhu H, Xu Y, Sui X, et al. Hematologic and immunological characteristics of Henoch-Schonlein purpura in rat and rabbit models induced with ovalbumin based on type III hypersensitivity. Scientific Reports. 2015;5:8862. Available at: http://www.nature.com/srep/2015/150309/srep08862/full/srep08862.html.
8. Gertz EM, Agarwala R, Mage RG, Schäffer AA. Comparative analysis of genome sequences of the Th2 cytokine region of rabbit (Oryctolagus cuniculus) with those of nine different species. Immunol Immunogenet Insights. 2011;3:59-82. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3519392/.
9. Shang H, Cao XL, Wan YJ, Meng J, Guo LH. IL-4 Gene Polymorphism May Contribute to an Increased Risk of Atopic Dermatitis in Children. Dis Markers. 2016;2016:1021942. Available at: https://www.hindawi.com/journals/dm/2016/1021942/.
10. Alizadeh-Navaei R, Rafiei A, Hedayatizadeh-Omran A, Mohammadzadeh I2, Arabi M. Ann Med Health Sci Res. 2014, Nov;4(6):837-40. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/25506473/.
11. Wu Z, Qin W, Zeng J, Huang C, Lu Y, Li S. Association Between IL-4 Polymorphisms and Risk of Liver Disease: An Updated Meta-Analysis. Medicine (Baltimore). 2015;94(35):e1435. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/2633 4904/.
12. Wang T, Tian L, Gao M, Song H, Wei Y, Xue Y. Interleukin (IL)-4 -590C>T polymorphism is not associated with the susceptibility of gastric cancer: An updated meta-analysis. Ann Med Surg. 2016, May;9:1-5. Available at: https://linkinghub.elsevier. com/retrieve/pii/S2049-0801(16)30054-1.
13. Potaczek DP, Kabesch M. Current concepts of IgE regulation and impact of genetic determinants. Clin Exp Allergy. 2012, Jun;42(6):852-71. Available at: http://dx.doi.org/10.1111/j.1365-2222.2011.03953.x.
14. Vychodilova L, Matiasovic J, Bobrova O, Futas J, Klumplerova M, Stejskalova K, et al. Immunogenomic analysis of insect bite hypersensitivity in a model horse population. Vet Immunol Immunopathol. 2013, Abr;152(3-4):260-8. Available at: https://linkinghub.elsevier.com/retrieve/pii/S0165-2427(12)00450-3.

Dynamics of the regulatory network of lymphocytes

Luis Mendoza

Instituto de Investigaciones Biomédicas, UNAM, Ciudad de Mexico, Mexico
lmendoza@biomedicas.unam.mx

Keywords: Systems Biology, dynamical systems, lymphocyte differentiation, regulatory network, T and B lymphocytes

There is a large quantity of information regarding the differentiation of lymphocytes at the molecular and cellular levels. Despite the wealth of information, there is no consensus regarding the structure and dynamical behavior of the regulatory network controlling the differentiation of lymphocytes. Regulatory networks are constituted by a set of molecules or molecular complexes and the regulatory interactions among them. These interactions may be either directly conveyed by means of a physical molecular interaction, or may be elicited via (possibly unknown) intermediaries. Regulatory networks allow for a limited number of asymptotic behaviors, which correspond to the observed stationary expression or activation patterns of the molecules that constitute the network. The modeling of regulatory networks as dynamical systems has shown to be an adequate approach to understand the molecular mechanisms integrating several types of signals that control cell differentiation processes. The presentation will focus on a regulatory network model that is able to qualitatively describe the expression patterns observed in CLP, pro-B, B naive, PC, DP, CD4+ naive, Th1, Th2, Th17, Treg, CD8+ naive, and CTL cells; under wild type and mutant backgrounds. Moreover, the model recovers the branching process leading from the pluripotent CLP to fully differentiated effector cells.

Modeling the multi-modular network of normal B lymphopoiesis blockage in acute leukemias

Jennifer Enciso[1,2,3], Elena R. Alvarez-Buylla[3,4], Luis Mendoza[5], Rosana Pelayo[1]

[1]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
[1]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[3]Center for Complexity Sciences, National Autonomous University of Mexico, Mexico City, Mexico
[4]Institute of Ecology, National Autonomous University of Mexico, Mexico City, Mexico
[5]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
jenny_gr8@msn.com; eabuylla@gmail.com; lmendoza@biomedicas.unam.mx; rosanapelayo@gmail.com

Keywords: Gene Regulatory Networks, Leukemia, Lymphoid, Tumor Microenvironment, CXCL12, Pro-inflammation

Cell fate decisions throughout the hematopoietic differentiation pathway depend on intrinsic factors and extrinsic signals provided by the bone marrow (BM) microenvironment where they reside. Abnormalities in the composition and function of hematopoietic niches have been proposed as key contributors of acute lymphoblastic leukemia (ALL) progression. Thence, leukemogenesis appear to be an emergent event of deregulated biological modules involved in determination of cellular processes (signal-transduction molecules, proliferation and apoptosis), genetic regulatory networks of early B lymphopoiesis (differentiation factors) and intercommunication elements with the BM microenvironment (chemokine receptors and adhesion molecules, among others). Our previous findings strongly suggest that pro-inflammatory cues may be detrimental to normal hematopoiesis while contributing to a CXCL12/CXCR4 disruption-related maintenance of ALL precursor cells within the BM.

By developing and simulating a dynamic Boolean mathematical system modeling the signaling networks between hematopoietic progenitor cells (HPC) and mesenchymal stromal cells (MSC) in the BM, we predicted that a temporal signaling from Toll like receptors (TLRs) ligation that results in NF-kB induction, is sufficient to induce instability of the CXCR4/CXCL12 and VLA4/VCAM1 interactions. Therefore, aberrant expression of NF-κB induced by intrinsic or extrinsic factors may contribute to create a tumor microenvironment where a negative feedback loop inhibiting CXCR4/CXCL12 and VLA4/VCAM1 cellular intercommunication axes allows the replacement of normal hematopoiesis by malignant cells. Considering that B-cell precursor acute lymphoblastic leukemia is the most common cause of death of pediatric cancer, our findings inspire the reconstruction of hybrid models composed by a gene regulatory network (GRN) for early B cell differentiation simulated as a boolean model, and a

signaling pathway continuous model with representative molecules involved in the transduction of microenvironmental cues. The GRN includes the main transcriptional factors directing the early stages of B cell differentiation, initiating with the lymphoid cell priming mediated by the expression of Runx1 and ending with the second peak of Rag expression that determines the maturation of the pre-BCR. Most of the transcription factors involved in this early differentiation stages have been reported to appear mutated in leukemic patients, however the results are very heterogeneous and they do not explain their participation in the remodeling of the BM leading probably, to the ablation of normal hematopoietic cells. Together, with the combined modeling of the GRN and the signaling pathways regulating HPCs responses to microenvironmental cues, we may provide an in silico tool to understand basic processes leading to the disruption of normal hematopoiesis and malignant B-ALL development. Information from the computational simulations will allow us the further identification of destabilizing molecules involved in pathocellular phenotypes endowed with hyperproliferative characteristics in differentiation blockage settings.

Regulatory network showcases mechanisms underling CD4+ T cell differentiation and plasticity

Mariana Esther Martinez-Sanchez[1,2], Elena R. Alvarez-Buylla[1,2]

[1]Center for Complexity Sciences, National Autonomous University of Mexico
[2]Institute of Ecology, National Autonomous University of Mexico
mar.esther23@gmail.com; eabuylla@gmail.com

keywords: plasticity, CD4+ T cells, regulatory T cells, chronic inflammation, TGF-beta, Boolean networks

To protect the organism, the immune system needs to be able to mount a robust response to control pathogens, adapt as the immune challenge changes, and regulate itself to avoid autoimmune responses. CD4+ T cells modulate the immune response. These cells are plastic, once differentiated they can alter their expression profile in response to changes in the environment [1].

Here we use a dynamical regulatory network to study CD4+ T cell differentiation and plasticity [2]. The model integrates transcription factors, signaling pathways, autocrine and exogenous cytokines and recovers both naive, effector (Th1, Th2, Th17, Th9, and Tfh) and regulatory (Treg, Tr1, Th3) cell types [3-7].

To study the plasticity of the system, we transiently perturb the value of the nodes and determine whether the perturbation leads to a transition between cell types. The model recovers the observed differentiation and plasticity of CD4+ T cells [2]. This methodology allows us to determine if there is a transition between two cell types and which interventions are necessary to induce this transition. Furthermore, we can use this approach to determine the secondary effects of the perturbation in other cell types.

The model also shows that the exogenous cytokines present in the microenvironment constrain which cell types and transitions are possible. In environments that promote effector cell types, most of the cell types recover correspond to effector cells and the transitions are towards this cell types. At the same time, in environments that promote regulatory cell types, the cell types recover correspond to regulatory cells and the transitions are towards this cell types. However, in the pro-Treg environment where exogenous IL-2 and TGF-B are present, there is a coexistence of regulatory and Th17 cells, which could prove insights into chronic inflammation [8].

To understand the mechanisms that underlie CD4+ T cell plasticity we study the regulatory interactions between cell types. Our results show the critical role of cytokines that regulate more than one cell type (TGFB, IL-2, and IL-10). We also show how the redundancy in the inhibitory regulations at the transcription factor and signaling pathway level affect the stability of the different cell types.

Complex properties like plasticity and robustness are the result of systems that integrate multiple signals and regulatory levels. The model and methodology presented here may be a useful framework to study other plastic systems and guide therapeutic approaches to immune system modulation.

REFERENCES

[1] DuPage, M., & Bluestone, J. A. (2016). Harnessing the plasticity of CD4(+) T cells to treat immune-mediated disease. Nat Rev Immunol, 16(3), 149–163. https://doi.org/10.1038/nri.2015.18

[2] Martinez-Sanchez, M. E., Mendoza, L., Villarreal, C., & Alvarez-Buylla, E. R. (2015). A Minimal Regulatory Network of Extrinsic and Intrinsic Factors Recovers Observed Patterns of CD4+ T Cell Differentiation and Plasticity. PLoS Computational Biology, 11(6), e1004324. https://doi.org/10.1371/journal.pcbi.1004324

[3] Zhu, J., & Paul, W. E. W. (2010). Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors. Immunological Reviews, 238(1), 247–262. https://doi.org/10.1111/j.1600-065X.2010.00951.x.Peripheral

[4] Vinuesa, C. G., Linterman, M. A., Yu, D., & MacLennan, I. C. M. (2016). Follicular Helper T Cells. Annual Review of Immunology, 34(1), annurev-immunol-041015-055605. https://doi.org/10.1146/annurev-immunol-041015-055605

[5] Schmitt, E., Klein, M., & Bopp, T. (2014). Th9 cells, new players in adaptive immunity. Trends in Immunology, 35(2), 61–8. https://doi.org/10.1016/j.it.2013.10.004

[6] Chihara, N., Madi, A., Karwacz, K., Awasthi, A., & Kuchroo, V. K. (2016). Differentiation and Characterization of Tr1 Cells. Current Protocols in Immunology, 113(April), 3.27.1-3.27.10. https://doi.org/10.1002/0471142735.im0327s113

[7] Travis, M. a, & Sheppard, D. (2014). TGF-β activation and function in immunity. Annual Review of Immunology, 32(November 2013), 51–82. https://doi.org/10.1146/annurev-immunol-032713-120257

[8] Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Lee, J., … Mathis, D. (2009). Fat Treg cells: a liaison between the immune and metabolic systems. Nature Medicine, 15(8), 930–939. https://doi.org/10.1038/nm.2002.Fat

The regulatory network controlling DCs differentiation

Mauricio Pérez-Martínez, Luis Mendoza

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
mauricio_pm@ciencias.unam.mx; lmendoza@biomedicas.unam.mx

Keywords: Dendritic Cells, Gene Regulatory Networks, Hematopoiesis, Systems Biology, differentiation

Dendritic cells (DCs) are hematopoietic cells that recognize, process and present antigens. DCs originate from a common myeloid progenitor or from a common lymphoid progenitor, while mature cells can be classified into at least three main groups: Classical or conventional DCs (cDCs), plasmacytoid DCs (pDCs) and Langerhans cells (LCs). The main molecules involved in the differentiation process of these cells are well characterized, including FLT3L, PU.1, IRF4, IRF8, and BCL6. However, the precise nature of the regulatory interaction among these and other key molecules remains unknown.

The recovery of the molecular regulatory network controlling the differentiation of DCs will be key to understand the molecular basis determining the ontogeny of these cells. To this aim, we inferred the regulatory network controlling the differentiation of splenic CD8+ cDCs using published experimental data, and studied its behavior by modelling it as a Boolean regulatory network. The steady states of the model correspond to the qualitative gene expression patterns reported in the literature for these cells, both under wild-type and mutant backgrounds. Specially, the model reproduces the molecular expression patterns corresponding to CDP, pre-cDC, pre-cDC1 and cDC CD8+ cells. Furthermore, perturbation analyzes of the model show that extracellular signals are always required, being either FLT3L or by GM-CSF. Also, the model shows that the commitment to pDC or cDC, is regulated in a feedback way between ID2 and E2-2.

Meta-analysis of the macrophage migration inhibitory factor -173 G/C and -794 CATT5-8 polymorphisms: Geographical and pathophysiological association with disease

Oscar Illescas[1], Juan Carlos Gomez Verjan[2], Lizbeth García-Velázquez[3], Imelda Juarez-Avelar[1], Miriam Rodriguez-Sosa[1]

[1]Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico

[2]Instituto Nacional de Geriatría, Mexico City, Mexico

[3]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico

oscarillescas@comunidad.unam.mx; carlosverjan@comunidad.unam.mx; lizgarvel@gmail.com; imelda_juarez@yahoo.com; rodriguezm@unam.mx

Keywords: Autoimmune Diseases, Inflammation Mediators, Polymorphism, Genetic, meta analysis, infectious diseases, MIF

Human macrophage migration inhibitory factor (MIF) is a cytokine that plays a role in several metabolic and inflammatory processes. MIF polymorphisms located on the gene promoter sequence had been associated with numerous diseases, such as arthritis and cancer. The MIF polymorphisms with more case-control studies reported are the short tandem repeat (STR) of the sequence CATT5-8 (rs5844572) and the single nucleotide polymorphism (SNP) G/C (rs755622), located at position -794 and -173 respectively. However, most reports concerning the association of MIF with pathologies are inconsistent and remain controversial. Therefore, we performed an innovative meta-analysis from 96 case-control studies on -173 G/C and -794 CATT5-8 MIF polymorphisms. For this, we stratified the data according to the subjects geographic localization or the disease pathophysiology, in order to give a more meaningful significance to the association to the polymorphism.

The SNP was strongly associated with an increased risk in autoimmune-inflammatory, infectious and age-related diseases subdivisions on the dominant and the recessive models; while arthritis presented significant association in the dominant and homozygous models, and cancer only in the dominant model. Inflammatory bowel diseases presented no significant association in any genetic model. Afterwards, we implemented a simple version of a network meta-analysis to compare the association of the polymorphism for two different subdivisions. We found a stronger association of the polymorphism for autoimmune than for age-related or autoimmune-inflammatory diseases subdivisions, and stronger association for infectious than for the autoimmune-inflammatory diseases subdivision. Also, we found clear differences in the

allele and genotype frequencies of both polymorphism when subjects were stratified according to geographic localization. Interestingly, Asia, Europe and Latin America subdivisions presented significant association to disease in the dominant and over-dominant models.

We report for the first time, meta-analyses of rs755622 and rs5844572 polymorphisms with a variety of stratified diseases and populations. Our results suggest that the polymorphisms association to disease varies according to the pathophysiological characteristics of the disease; also, the allelic and genotypic frequency of the rs5844572 STR is reported for the first time including populations from Africa, Asia, Europe, Middle-East, Latin-America and North-America. Further experimental validation is needed to confirm the associations found in this study, and hence, results may help direct future research on MIF -173 G/C and -794 CATT5-8 in diseases in which the relation is clearer and thus assist the search for more plausible applications.

Diabetes type 2 as an inflammatory disease: A complex network analysis

María Barrera, Marcia Hiriart, Germinal Cocho, Carlos Villarreal

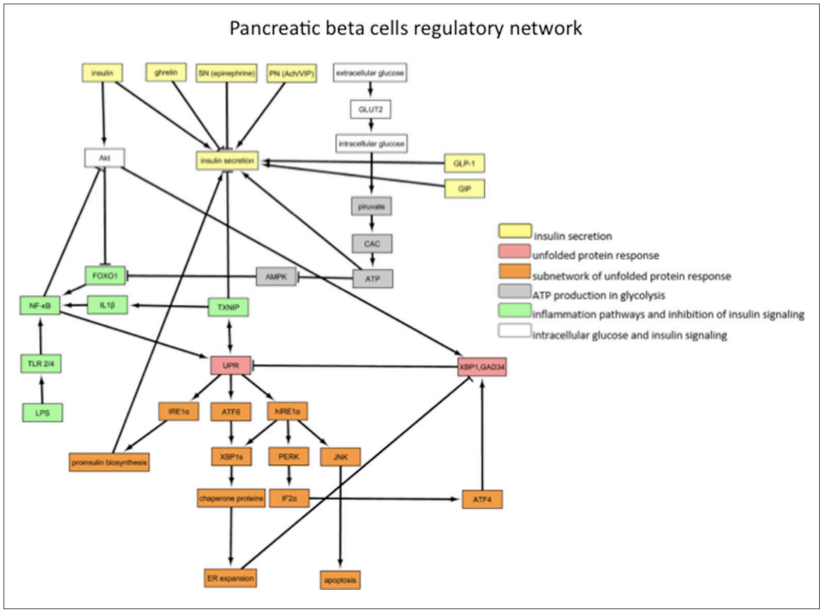
Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
marybaesp@yahoo.com.mx; mhiriart@ifc.unam.mx; germinal.cocho@gmail.com; carlos@fisica.unam.mx

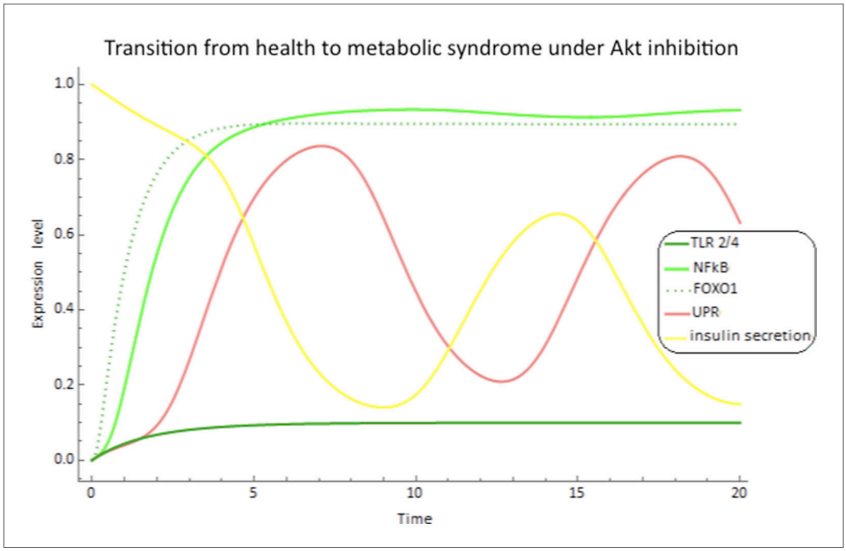
Keywords: metabolic syndrome, complex networks, diabetes type 2, Inflammatory diseases, Txnip

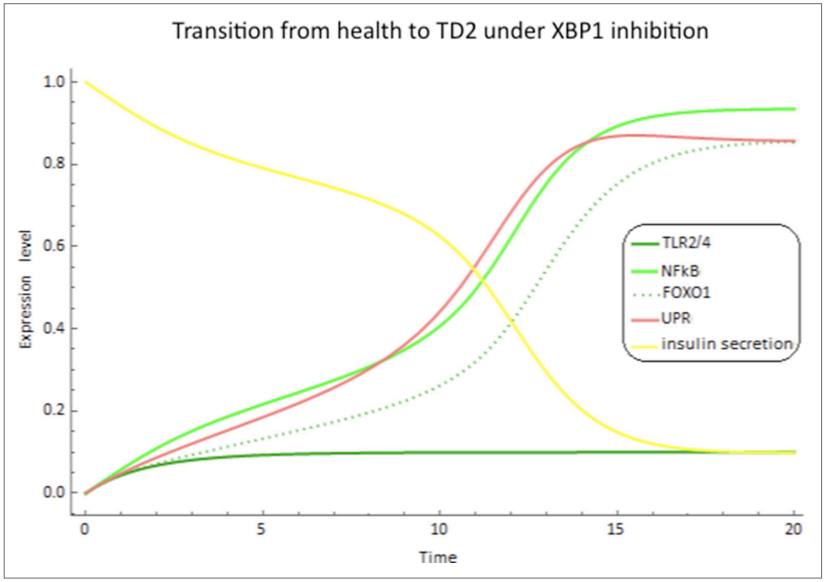
Type 2 diabetes (T2D) is a complex disease that comprises a group of metabolic disorders characterized by a steady decline in insulin secretion from pancreatic beta cells, resulting in elevated glucose levels that cause long-term complications in patients leading to eye, nerve, and kidney disfunction [1]. T2D is usually preceded by a set of altered elements, in the process known as metabolic syndrome (MS), which includes central obesity, high blood pressure, dyslipidemia, glucose intolerance, and insulin resistance [2].

In this work we analyze relations between essential factors involved in the development of MS and TD2 by considering components involved in insulin signaling, such as metabolic and inflammatory pathways [3]. The analysis considers a complex regulatory network for pancreatic beta cells that integrates stimulatory and inhibitory interactions between cellular elements responsible of glucose internalization, processing, and storage. Every network node may represent a gene, a transcription factor, a cytokine, etc., while their links represent relative interactions. These interactions induce dynamical processes that with the course of time modify the initial expression levels of the network nodes, eventually leading to altered equilibrium regimes characterized by activation and inhibition patterns exhibited by cell components. The main expression patterns are identified as states of health, MS, or TD2. Naturally, the boundary between these states is diffuse and a number of hybrid patterns may also develop. Subsequently, transitions between health, MS, and TD2 are simulated by introducing modifications of characteristic expression times (or decay rates) of the network components. This is equivalent to considering exhaustion mechanisms of cellular components, the action of overnutrition, chronic inflammation, over-expression of endoplasmic reticulum stress, etc.

Usually, regulatory network interactions are formalized in terms of dichotomic logic propositions satisfying Boolean rules, with expression values 0 (inhibited), or 1 (activated). In this work we consider a more general continuous approach [4] based on fuzzy logic [5], where the expression level of each node may acquire any value within the


Pancreatic beta cells regulatory network
insulin
ghrelin
SN (epinephrine)
PN (Ach/VIP)
extracellular glucose
GLUT2
intracellular glucose
Akt
insulin secretion
GLP-1
GIP
pirvate
CAC
ATP
AMPK
FOXO1
NF-κB
IL1β
TXNIP
TLR 2/4
LPS
UPR
XBP1,GADD34
IRE1α
ATF6
hIRE1α
proinsulin biosynthesis
XBP1s
PERK
JNK
chaperone proteins
IF2α
ATF4
ER expansion
apoptosis
insulin secretion
unfolded protein response
subnetwork of unfolded protein response
ATP production in glycolysis
inflammation pathways and inhibition of insulin signaling
intracellular glucose and insulin signaling


Transition from health to metabolic syndrome under Akt inhibition
Expression level
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
Time
TLR 2/4
NFkB
FOXO1
UPR
insulin secretion



interval [0,1], so that the degree to which an object exhibits a given property is specified by a characteristic distribution function ranging between completely false (0), up to completely true (1). The system dynamics is then described by a set of ordinary differential equations for the rate of change of the expression level of each node. This rate is determined by the balance between an expression source, represented by the characteristic function, and a sink given by the node´s decay rate. The states resulting form the network dynamics may be identified with different stages involved in the transit from health to TD2.

We observed that several nodes play a primordial role in the development of MS and TD2. We identified a module composed by four nodes: Akt (protein kinase B), XBP1 (X-box binding protein 1 splicing), TXNIP (Thioredoxin-interacting protein), and UPR (unfolded protein response) which acts as a switching device leading the transition between the disease stages. Particularly, strong inhibition of Akt and XBP1 leads to TD2, while strong inhibition of TXNIP leads to amelioration of a patient´s condition. Recent reports show the central role played by over-expression of TXNIP in TD2 development, suggesting the relevance of investigating therapies aimed to inhibit TXNIP expression [6,7].

REFERENCES

[1] De Fronzo et al., Type 2 diabetes mellitus,Nature Reviews. Disease Primers. 2015;1:1-22

[2] Lusis AJ, Attie AD, Reue K. Metabolic syndrome: from epidemiology to systems biology. Nature reviews. Genetics [Internet]. 2008;9:819–30. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2829312&tool=pmcentrez&rendertype=abstract

[3] Dandona P. Inflammation: the link between insulin resistance, obesity and diabetes. Trends in Immunology. 2004;25:4–7.

[4] Villarreal C, Padilla-Longoria P, Alvarez-Buylla ER. General theory of genotype to phenotype mapping: Derivation of epigenetic landscapes from n-node complex gene regulatory networks. Physical Review Letters. 2012;109:1–5; Davila-Velderrain J, Villarreal C, Alvarez-Buylla ER. Reshaping the epigenetic landscape during early flower development: induction of attractor transitions by relative differences in gene decay rates. BMC systems biology [Internet]. 2015;9:20. Available from: http://www.biomedcentral.com/1752-0509/9/20

[5] TJ Ross, Fuzzy Logic with Engineering Applications, Third Edition, 2010 Wiley Online Library, Online ISSBN: 9781119994374

[6] G Xu, J Chen, G Jin, and A Shalev, Thioredoxin-interacting protein regulates insulin transcription through micro RNA-204. Nature Medicine. 2013; 19: 1141-1147

[7] A Shalev, Minireview: Thioredoxin-interacting protein: regulation and function in beta pancreatic cells. Mol. Endocrin. 2014; 8: 1211-1220

Model for the study of hipersensitivity to drugs based on bucillamine and the HLA-DRB1*08:02 allele in Colombian indigenous populations

Carlos Hernando Parga Lozano, José Eliecer Brito Alvarez, Mateo De Las Salas Tirado, Alexander David Tapia Sierra

Universidad Libre de Colombia Seccional Barranquilla, Barranquilla, Colombia
pargacarlos@yahoo.com; joseeliecerb@hotmail.com; mdelassalas020@estunilibrebaq.edu.co; alex_sierra12@hotmail.com

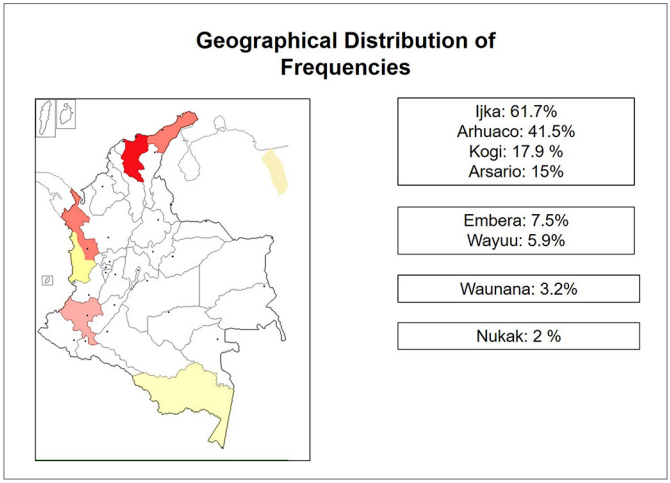
Keywords: HLA, allergies, Amerindian, Bucillamine, Hyper sensibility

Introduction: Allergic diseases and hypersensitivity reactions are common disorders that in turn consist of an extensive genetic component in which the molecules of the Major Histocompatibility Complex are included, which have certain alleles associated with the development of hypersensitivity to certain drugs, among which is the allele HLA-DRB1 * 08: 02 as a predisposing factor of hypersensitivity to Bucillamine (drug indicated in Rheumatoid Arthritis). This drug is the starting point for the study of the relationship between hypersensitivity reactions to medications and the expression of certain alleles of CMH.

Objective: Find the relationship between hypersensitivity to the drug and the expression of the specific allele in Amerindian populations of the Sierra Nevada de Santa Marta and in turn suggest the application of the methodological model proposed in similar studies that seek to relate drug allergies with specific HLA alleles

Methodology: A systematic search of information was carried out in the Sience, ScienceDirect, Elsevier and Pubmed databases, the frequencies obtained were tabulated and organized according to their expression to be analyzed with the MEGA7 software.

Results: A significant frequency of the HLA-DRB1 * 08: 02 allele was found in the Ijka (61.7%), Arhuaco (41.5%), Kogi (17.9%) and Arsario (15%) populations. Which were positive for the Rheumatoid Arthritis marker HLA-DRB1 * 04: 07



Bucillamine Allergy

Allele	Population	Allele Frequency	Sample Size
*DRB1*08:02*	Colombia Sierra Nevada de Santa Marta Ijka pop 2	0.6170	30
*DRB1*08:02*	Colombia Sierra Nevada de Santa Marta Arhuaco	0.4150	107
*DRB1*08:02*	Colombia Sierra Nevada de Santa Marta Kogi	0.1790	42
*DRB1*08:02*	Colombia Sierra Nevada de Santa Marta Arsario	0.1500	18
*DRB1*08:02*	Colombia Sierra Nevada de Santa Marta Kogi pop 3	0.1290	31

Rheumatoid Arthritis Predisposition

Allele	Population	Allele Frequency	Sample Size
*DRB1*04:07*	Colombia Sierra Nevada de Santa Marta Kogi pop 3	0.6130	31
*DRB1*04:07*	Colombia Sierra Nevada de Santa Marta Kogi	0.5520	42
*DRB1*04:07*	Colombia Sierra Nevada de Santa Marta Arsario	0.4500	18
*DRB1*04:07*	Colombia Jaidukama	0.3270	39
*DRB1*04:07*	Colombia West Embera	0.2000	20
*DRB1*04:07*	Colombia Sierra Nevada de Santa Marta Arhuaco	0.1630	107
*DRB1*04:07*	Colombia West Waunana	0.1500	30
*DRB1*04:07*	Colombiaa Northwest Tule	0.1210	29
*DRB1*04:07*	Colombia Guajira Peninsula Wayuu	0.1070	88
*DRB1*04:07*	Colombia Bogota And Medellín Mestizo	0.1000	65

Conclusion: A careful management of Bucillamine and drugs molecularly similar to this in susceptible Amerindian populations is recommended, in turn it is suggested the use of the proposed model for the study of other drugs that can trigger allergic reactions based on the expression of alleles of the HLA.

Mathematical modelling of complex host-pathogen interactions on epithelial tissues

Elisa Domínguez-Hüttinger

Universidad Nacional Autónoma de México, Morelia, Mexico
elisa.dominguez@mail.ecologia.unam.mx

Keywords: Systems Biology, Host pathogen interactions, mathematical modelling, mucosal immunity, Treatment design

The body is constantly in contact with a myriad of different microorganisms, including virulent bacterial strains that can cause infection as they come in contact with normally sterile tissues, and commensal organisms that can, under pathogenic conditions, elicit aberrant host responses leading to tissue damage or infection. Many different host responses, such as immune system activation and tissue remodelling, counteract these microbial challenges, and are responsible for the healthy, homeostatic co-existence between the host and the microorganisms. However, many genetic and environmental factors including mutations, polymorphisms, previous infectious events, or weakened immune responses, can disrupt these complex interactions between the microorganisms and the host. Such aberrant host responses underlie a whole spectrum of diseases, comprising several of life-threatening, severe pathological conditions such as invasive infections or chronic tissue damage which can result from unresolved inflammatory responses to pathogens. Due to the complexity and diversity of these host-microorganism interactions, classical experimental approaches focusing on isolated aspects of the host response might often not be enough to understand the resulting pathogenic mechanisms. Recently, novel systems biology approaches have been proposed to represent and analyse such complex host-microorganisms interactions. These approaches allow the systematic integration and analysis of clinical and experimental data, and to perform rigorous and systematic assessments of the role of different risk factors on the onset and progression of pathogen-associated diseases. In this talk, I will show how such a theoretical framework can be used to formally represent and computationally and mathematically analyse the complex interactions between the host and the microorganisms. Focusing on host-pathogen interactions on the airway mucosa and on the epidermis, I will show mathematical models can be used to infer possible pathogenic mechanisms that result from impaired host-microorganism interactions, and to design optimal treatment strategies that counteract these pathogenic mechanisms in a minimally invasive and patient-specific way.

Immunostimulatory capacity of the Trypanosoma cruzi TcTASV protein family engineered with different adjuvants

Lucas D. Caeiro[1,2], Yamil E. Masip[2], Lorena M. Coria[1,2], Mariana Rizzi[1,2], Matías E. Rodriguez[1,2], Guido Molina[3], Celeste V. Pueblas Castro[2], Daniel O. Sánchez[1,2], Juliana Cassataro[1,2], María P. Molinari[1,3], Valeria Tekiel[1,2]*

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]Institute of Biotechnological Research "R.Ugalde" (IIB-Intech), National University of San Martin, Argentina
[3]Laboratorio de Baculovirus, Instituto de Biotecnología., INTA, Cordoba, Argentina
lucaniel@gmail.com; yemasip@gmail.com; lorena.coria@iibintech.com.ar; mai_r9@hotmail.com; exequiel198803@gmail.com; molina.guido@inta.gob.ar; celes1906@hotmail.com; dsanchez@unsam.edu.ar; jucassataro@iibintech.com.ar; molinari.maria@inta.gob.ar; vtekiel@gmail.com

Keywords: Vaccine, adjuvant, baculovirus, Protein Families, Trypomastigote, TcTASV, U-Omp19

Chagas'disease is an endemic illness caused by the protozoan parasite Trypanosoma cruzi, that affects more than 6 million people in Latin America. T. cruzi has a complex life cycle alternating between a reduviid insect and a mammalian host. Proteins differentially expressed on circulating bloodstream trypomastigotes and/or on intracellular amastigotes (stages present in the mammalian host) are those somehow involved in parasite survival. Most of these molecules are either virulence factors of the parasite or involved in immune system evasion, and, therefore, interesting targets for rational interventions, like vaccine design.

The Trypomastigote Threonine, Valine and Serine rich (TcTASV) is a medium-size T.cruzi multigene family that is present in all T. cruzi lineages and has no orthologs in other organisms. All TcTASVs have 3'UTRs as well as amino and carboxi-termini conserved. The variable central core of the proteins -along with sequence, protein length, and motifs- allows to distinguish 4 TcTASV subfamilies (A, B, C and W). TcTASV-A and TcTASV-C are the major subfamilies, with 15-20 members each one. Although all TcTASV are expressed into trypomastigotes and/or amastigotes, the pattern of expression oscillate among the different subfamilies. TcTASV-A is expressed intracellularly both in amastigotes and trypomastigotes, while TcTASV-C is solely expressed in trypomastigotes, at the cell surface and strongly secreted in extracellular vesicles (Garcia et al, 2010; Bernabó et al, 2013; and unpublished results). In line with our results, peptides from TcTASV-A and TcTASV-C were recently found in proteomes of bloodstream trypomastigotes and trypomastigote's secretomes of different

T. cruzi strains. Also previously, by means of genetic library immunization, we identified a fragment of a TcTASV-C gene among a pool of protective clones (Tekiel et al, 2009). Later, we evaluate TcTASV-C as an individual vaccine in a prime (DNA+GM-CSF) and boost (recombinant protein+AlOH) protocol. After challenged with a highly virulent T. cruzi strain (RA, DTU TcVI) vaccinated mice presented delayed appearance of bloodstream trypomastigotes but only slight improvement in survival rates. This fact can be related with the immune response triggered by the immunization scheme: a strong humoral but negligible cellular immune responses. Considering that both TcTASV-A and TcTASV-C subfamilies are in contact with the host immune system in vivo (Floridia et al, 2016), altogether with their expression dynamics and the previous vaccine results, in the present study we evaluated different immunization protocols employing both TcTASV-C and TcTASV-A and several delivery systems to trigger both humoral and cellular specific immune responses. In this context we hypothesize that an adequate vaccination protocol should induce a cellular response against TcTASV-A (intracellular) while a humoral response against TcTASV-C (surface and secreted) should be desirable.

The first vaccination protocol tested consisted in the administration of both recombinant TcTASV-C and TcTASV-A (rTcTASV-C+A) simultaneously adjuvanted with aluminum hydroxide and saponin. Male C3H/HeJ mice were immunized by s.c. route with 3 doses of 25μg of proteins (12,5 μg each) intervaled by 15 days. Splenocytes from TcTASV immunized animals produced IFNg upon restimulation with TcTASV-A, but not after restimulation with TcTASV-C. On the other hand, the humoral response elicited by vaccination was mainly against TcTASV-C and only slightly higher than controls against TcTASV-A. The anti-TcTASV-C antibody response showed a mixed Th1/Th2 pattern, with a bias to IgG2a>IgG1. A vaccination scheme employing rTcTASV-A+C delivered with aluminum and boosted with saponin gave similar results. We conclude that it is possible to trigger both arms of the immune response after vaccination with proteins from both subfamilies simultaneously or sequentially formulated with saponin and aluminum salts.

Then, we evaluate another immunization scheme employing a protease inhibitor from B. abortus with adjuvant capacity, the unlipidated outer membrane protein 19 (U-Omp19), in our model. U-Omp19 increases the antigen half-life in APCs improving cross-presentation and triggers Th1, Th17, CD8+ antigen specific responses (Coria et al, 2016). Mice were immunized by the s.c. route with 3 doses of rTcTASV-C+A (25μg each/mice) plus U-Omp19, or with rTcTASV-C+A (25μg each) simultaneously delivered with U-Omp19, saponin and aluminum hydroxide. Vaccinated mice developed anti-TcTASV-A as well as anti TcTASV-C specific antibodies. However the group solely adjuvanted with U-Omp19 presented lower titers than the group immunized with all adjuvants (TcTASV-A: 3200 vs >12800; TcTASV-C: 6400 vs >12800). Moreover,

although both groups of vaccinated mice presented lower bloodstream trypomastigotes in the early infection ($p<0.01$ vs sham vaccinated group) only the group vaccinated with U-Omp19+ saponin + aluminum controlled parasitemia up to 22 days after the challenge with a highly virulent T. cruzi strain.

Ultimately, we designed a recombinant baculovirus (BV) encoding a TcTASV-A gene to be displayed at the capsid, as vaccine delivery system. Mice immunized with OVA-derived peptides by the capsid display strategy elicited potent cytotoxic immune response (Molinari et al, 2011). Here, TcTASV-A was cloned to be expressed as a fusion protein to a second copy of VP39, the major nucleocapsid protein of budded and occlusion-derived virions. A 3xFLAG tag was included into the TcTASV-A-VP39 construct to accurately assess protein expression, which has been cloned under the control of the polyhedrin promoter of the pFastBac plasmid (Invitrogen). The plasmid was used to produce recombinant BVs using the Bac-to-Bac Baculovirus Expression System (Invitrogen), and viral progenies were propagated in Sf9 cells. Correct TcTASV-A-3xFLAG-VP39 expression was confirmed both by western blot and immunofluorescence. Recombinant BVs expressing TcTASV-A were titrated by end-point dilution and used to immunize mice along with rTcTASV-C protein. Cellular and humoral immune responses will be evaluated on mice receiving this vaccination schedule, which is currently ongoing.

As a whole, we conclude that immunization protocols with both TcTASV-A and TcTASV-C together with adjuvants that prompted different arms of the immune system improved the immunostimulatory capacity of TcTASVs. Moreover, including antigens expressed in different stages of the parasite integrated into a multistage vaccine, should provide –hopefully- a more efficient protection against a parasite challenge and a better infection outcome than the immunization with a vaccine that includes antigens from a single parasite stage.

REFERENCES

Tekiel V., Alba-Soto C., González Cappa S.M., Postan M. and Sánchez D.O. (2009). Identification of novel vaccine candidates for Chagas' disease by immunization with sequential fractions of a trypomastigote cDNA expression library. Vaccine. 27 (9): 1323- 1332.

García E., Ziliani M., Agüero F., Bernabó G., Sánchez D.O., Tekiel V. (2010). TcTASV: A novel protein family in Trypanosoma cruzi identified from a subtractive trypomastigote cDNA library. PLoS Neglected Tropical Diseases. 4 (10); pii: e841. doi: 10.1371/journal.pntd.0000841.

Bernabó G., Levy G., Ziliani M., Caeiro L.D., Sánchez D.O., Tekiel V. (2013). TcTASV-C, a protein family in Trypanosoma cruzi that is predominantly trypomastigote-stage specific and secreted to the medium. PLoS ONE. 8 (7): e71192. doi: 10.1371/journal.pone.0071192.

Floridia-Yapur N., Monje Rumi M., Ragone P., Lauthier J.J., Tomasini N., Alberti D'Amato A., et al. (2016). The TcTASV proteins are novel promising antigens to detect active Trypanosoma cruzi infection in dogs. Parasitology. 143 (11): 1382-9. doi: 10.1017/S0031182016000822.

Coria L.M., Ibañez A.E., Tkach M., Sabbione F., Bruno L., Carabajal M.V., et al. (2016). A Brucella spp. Protease Inhibitor Limits Antigen Lysosomal Proteolysis, Increases Cross-Presentation, and Enhances CD8+ T Cell Responses. J Immunol. 196 (10):4014-29. doi: 10.4049/jimmunol.1501188.

Molinari P., Crespo M.I., Gravisaco M.J., Taboga O., Morón G. (2011) Baculovirus capsid display potentiates OVA cytotoxic and innate immune responses. PLoS One. 6(8):e24108. doi: 10.1371/journal.pone.0024108.

A methodology to assess in vitro infectivity of Trypanosoma cruzi in human monocytes

Liliana Estefania Villanueva Lizama[1], Adriana Solis Angulo[1], Julio Vladimir Cruz Chan[1], Maria Jesus Ramirez Sierra[1], Miguel Enrique Rosado Vallado[1], Eric Dumonteil[1,2]

[1]Autonomous University of Yucatan, Mérida, Mexico
[2]Tulane University, New Orleans, United States
liliana.villanueva@correo.uady.mx; adrianasolis94@gmail.com; julio.cruz@correo.uady.mx; rsierra@correo.uady.mx; rvallado@correo.uady.mx; edumonte@tulane.edu

Keywords: Chagas Disease, Flow Cytometry, Trypanosoma cruzi, Vaccines, in vitro

The protozoan parasite Trypanosoma cruzi (T. cruzi) can infect any nucleated cell of mammalian host, evade the immune response and establish a systemic chronic infection causing Chagas disease. Chagasic cardiomyopathy is the mortal form of Chagas disease, occurs in 30% of infected people and there aren't effective treatments or vaccines to prevent it. In Mexico, the estimated prevalence is at least 0.65%, with 1.1 million people infected with. Trypomastigotes, the infective form of the parasite, entry to cells via phagocytosis and then they differentiate to the amastigote stadium (replicative intracellular form). In vitro models of infection are used to evaluate new trypanocidal drugs, vaccines or immunotherapies by quantification of infected cells and measuring of intracellular amastigotes. Manual or semi-automatic quantification of infected cells and amastigotes requires trained, is very subjective and takes much time. We developed a strategy to assay percentage of infection and measure indirectly parasite replication using a fluorescent marker and flow cytometry. Monocytes are highly susceptible to T. cruzi infection whereby where used as target cells to T. cruzi H1 strain infection. Trypomastigotes derived from THP-1 culture were labeled with V450 (violet proliferation dye). Briefly, 6.0 x 106 parasites were incubated with V450 (1 μM) for 10 min at 37°C. Labeled parasites were washed two times with cold PBS and then with RPMI plus 10% inactivated fetal bovine serum, by centrifugation at 1500g for 10 min at 25°C. The infection was performed using 10 parasites/cell. Cells and parasites were incubated in suspension at 37°C in 5% CO2 for 2 hours. After the incubation period the cells were washed two times by centrifugation with PBS at 500 x g for 10 min at 4°C to remove extracellular parasites. Cells were fixed with 4% formaldehyde solution and stores until be analyzed in a flow cytometer. Parasites not stained and RPMI cultures were used as controls. We found 30% of infected cells (THP-1 V450+) using flow cytometry, this methodology allow us to measure in vitro infection using a semi quantitative method. This approach will be helpful to evaluate antigen candidate vaccines against T. cruzi infections but also can be used to assay the effect of drugs and immunotherapies.

Expression of breast cancer-related epitopes targeting IGF-1 receptor, in chimeric human parvovirus B19 virus-like particles

Jorge Alberto Salazar-González[1], Alail Antonio Ruiz-Cruz[1], Ismael Bustos-Jaimes[1], Sergio Rosales-Mendoza[2], Leticia Moreno-Fierros[1]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico
jorge_fhkh@hotmail.com; biol.antonio.ruiz@gmail.com; ismaelb@unam.mx; rosales.s@fcq.uaslp.mx; lemofi@unam.mx

Keywords: Parvovirus B19, Human, breast cancer, IGF-I receptor, Recombinant vaccine, Virus-like particle (VLP)

Virus-like particles (VLPs) are nanoparticles comprised of highly ordered repetitive structures, macromolecular assemblies of viral proteins without carrying infectious genomic material, which effectively crosslink B-cell receptors (Bachmann et al., 1993). VLPs can stimulate both innate and adaptive immune responses, aided by their particulate structure which favors uptake by antigen presenting cells (Keller et al., 2010). They thus combine both safety and the potency to elicit a strong immune response (Grgacic & Anderson 2006, Kushnir et al 2012) due to their size, shape and surface where they display epitopes in a dense repetitive array (Bachmann & Jennings 2010, Chen & Lai 2013). These intrinsic immunological characteristics of VLPs are augmented by their self-adjuvant properties and demonstrated success as delivery system products. Indeed, up to date, there is plenty of reports sustaining their potential utility of VLP based-antigen derived systems in vaccine development field.

Herein we set out to design a chimeric breast cancer VLP-based vaccine. As a model we selected the expression of self-assembly VLPs from the Human parvovirus B19 (B19), derived from the recombinant VP2 protein expressed in Escherichia coli (Sánchez-Rodriguez et al. 2012) fused to 4 epitopes of the insulin-like growth factor-1 receptor (IGF-1R).

IGF-1R participates in cell growth, also it plays an important role in the metastatic process regulating cell migration, invasion and angiogenesis. IGF-1R has been associated with angiogenesis in several tumor types since its activation increases the expression of VEGF. In patients with metastatic breast cancer, an elevated level of serum IGF-1 and VEGF has been observed, compared with controls and patients without metastatic disease (Coskun et al, 2003). Inhibition of IGF-1R in several tumor lines produced a decrease in VEGF expression, angiogenesis and metastasis (Reinmuth et al, 2002). A bioinformatic report

identifying B epitopes reveals 2 linear epitopes linear epítopes reported by Bayrami et al. 2016, P4 (CRHYYYAGVCVPACPP) and P8 (RQPQDGYLYRHNYCSK). Also two relevant epítopes: 249 (GDLTNRCTMEEKPMEK) and 86 (KMCPSTENNESAPDNDT) that bind to both MHC class 1 and 2, with higher affinity for; 249: HLA-DR4, HLA-DR3 and HLA-DR2; 86: HLA-DR4, HLA-DP2 and HLA-DR3 (Mahdavi et al. 2017).

Cancer peptide vaccines produce antitumor response by activation of CD4+ in association with presentation of antigens in the surface of the cells by major Histocompatibility Complex (MHC) class I or II molecules. The characteristic of these epitopes of being able to bind MHC molecules type 1 and 2 is attractive to generate immune responses of cellular and humoral type, making it attractive to complement the anti-tumor immune response.

In this report we developed a self-assembly versatile VLP expression system, to display the IGF-1R epítopes described above in different configurations and the citotoxic potential and humoral responses of chimeric VLPs will be determined to assess the most efficient vaccine prototype configuration against breast cancer (Figure 1).

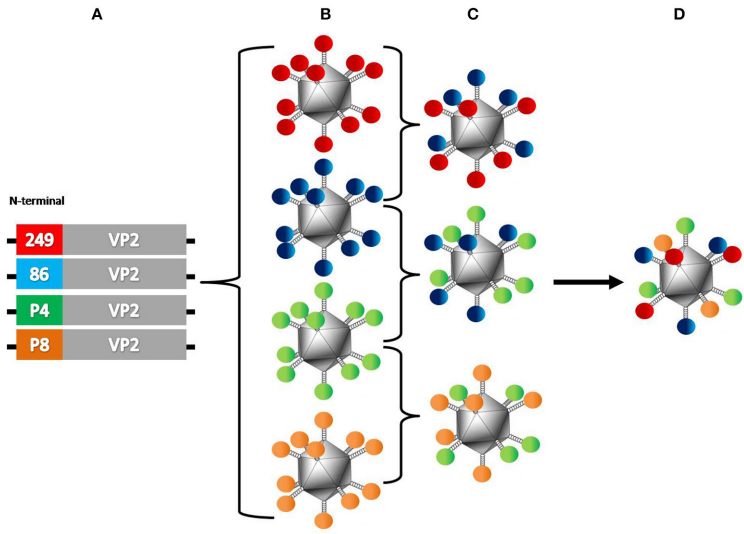


FIGURE 1: Expression strategy and possible assembly conformations for IGF-1R epitopes-VP2 VLPs. Each IGF-1R epitopes, 249,86,P4, and P8 were fused separately into the *Human parvovirus* B19 VP2 (A), to create a collection of several subunits of chimeric VP2. VLPs self-assembly can produce a versatile variety of configurations: a full assembly from a single epitope (B), two or more different epitopes in a single assembled VLP, to achieve a multiepitope versatile configuration displaying each epitope in the surface of the VLPs (C,D).

To date two recombinant clones of B19 VP2 expressing the 249 and the P8 epitope has been expressed and next step is to assemble VLPs. These can be under a full 249 or P8 VLPs of a mixure of both 249/P8 VLP to create a multiepitope VLP-approach.

This will allow a promising a versatile vaccine prototype that can be used as immunotherapy to other cancer cells.

REFERENCES

Bachmann, M.F., Rohrer, U.H., Kündig, T.M, et al., (1993). The influence of antigen organization on B cell responsiveness. Science. 262,1448–1451. doi: 10.1126/science.8248784.

Keller, S.A., Schwarz, K, Manolova, V., et al., (2010). Innate signaling regulates cross-priming at the level of DC licensing and not antigen presentation. Eur J Immunol. 40,103-112. doi: 10.1002/eji.200939559.

Grgacic, E.V., and Anderson D.A. (2006). Virus-like particles: passport to immune recognition. Methods. 40, 60-65

Kushnir, N., Streatfield, S.J., and, Yusibov, V. (2012). Virus-like particles as a highly efficient vaccine platform: diversity of targets and production systems and advances in clinical development. Vaccine. 31, 58-83.

Bachmann, M.F., and Jennings, G.T. (2010). Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. Nat Rev Immunol. 10, 787-796.

Chen, Q., and Lai, H. (2013). Plant-derived virus-like particles as vaccines Hum Vaccin Immunother, 9, 26-49

Sánchez-Rodríguez, S.P., Münch-Anguiano, L., Echeverría, O., et al., (2012). Human parvovirus B19 virus-like particles: In vitro assembly and stability. Biochimie. 94, 870-878. doi: 10.1016/j.biochi.2011.12.006.

Coskun, U., Gunel, N., Sancak, B., et al., (2003). Significance of serum vascular endotelial growth factor, insulin-like growth factor-I levels and nitric oxide activity in breast cancer patients. Breast. 12,104-110.

Reinmuth, N., Fan, F., Liu, W., et al., (2002) Impact of insulin-like growth factor receptor-I function on angiogenesis, growth, and metastasis of colon cancer. Lab Invest. 82, 1377–1389

Bayrami, V., Keyhanfar, M., Mohabatkar, H., et al., (2016). In silico prediction of B cell epitopes of the extracellular domain of insulin-like growth factor-1 receptor. Mol Biol Res Commun. 5, 201-214.

Mahdavi, M., Moreau, V., and Kheirollahi, M. (2017). Identification of B and T cell epitope based peptide vaccine from IGF-1 receptor in breast cancer. J Mol Graph Model. 75:316-321. doi: 10.1016/j.jmgm.2017.06.004.

Development of bioluminescent H1 T. cruzi for evaluation of tissue specific immune response

Kristyn Amber Hoffman[1,2], Liliana Villanueva-Lizama[1,2,3], John Kelly[4], Peter Hotez[1,2], Maria Elena Bottazzi[1,2], Kathryn Jones[1,2], Julio Vladimir Cruz-Chan[1,2,3]

[1]Texas Children's Hospital Center for Vaccine Development, Houston, United States
[2]Baylor College of Medicine, Houston, United States
[3]Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autonoma de Yucatan, Merida, Mexico
[4]London School of Hygiene & Tropical Medicine, London, United Kingdom
kristyn.hoffman@bcm.edu; liliana.villanueva@bcm.edu; john.kelly@lshtm.ac.uk; hotez@bcm.edu; bottazzi@bcm.edu; kathrynj@bcm.edu; vladimir.cruz@correo.uady.mx

Keywords: Trypanosoma cruzi, Bioluminescence imaging, luciferase reporter assay, Firefly expression, Protozoan transfection

Acute Trypanosoma cruzi infection rarely manifests with clinical symptoms in humans, but is associated with high parasite burden in many tissues. One tissue of concern is the heart, where T. cruzi causes damage that can later manifest as severe cardiomyopathy. We have incorporated a gene for the luciferase enzyme into an H1 strain of T. cruzi. Upon exposure to the substrate, luciferin, the protein product of this gene creates a bioluminescent signal, which can be detected in live cells. The gene was amplified as a linear plasmid using a bacterial cloning system. The plasmid was incorporated into the parasite genome via electroporation technique. The transfected parasites were found to contain luciferin, as demonstrated with a functional assay and quantified by luminometer. The modified parasite strain was isolated from a human patient from an area in Mexico endemic with Chagas disease. We have used this strain to develop a murine model of Chagas that reliably recapitulates the three stages of infection, acute, indeterminate and chronic. Infected mice demonstrate relevant pathologic manifestation of the disease, characterized by progression of worsening cardiomyopathy. The histopathologic findings in the acute phase include intense inflammatory infiltrate into the heart. Using the bioluminescent mutant of this parasite, we had the ability to monitor the tissue specificity of the parasite with serial monitoring. Comparisons of the immune response in tissues between the acute phase and the chronic phase, with relevant parasite tissue specificity information was critical to understanding the role of the immune system in tissues with and without replicating parasites. These experiments shed light on the importance of parasite persistence in tissues other than the heart, as this may contribute to cardiac disease.

Design, expression and purification of two multi-epitopic vaccines for HIV-1

David Gilberto Pérez Saucedo, Leticia Moreno-Fierros, Alail Antonio Ruíz Cruz

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
xlv2008@gmail.com; lemofi@unam.mx; callesde@hotmail.com

Keywords: Biotechnology, HIV, Vaccines, Synthetic, E. coli, VP2 protein, Multiepitopic recombinant protein

Human Immunodeficiency Virus (HIV), member of the Retroviridae family is the causal agent of the Acquired Immunodeficiency Syndrome (AIDS). According to World Health Organization estimates, by the end of 2016 there were 36.7 million people living with HIV, of which 2 million were new infections, and 1.2 million people died from AIDS. Wide spread adoption of anti-retroviral therapy (ART) has improved life expectancy and life quality of infected individuals by means of control of viral replication. Despite its efficacy, the therapy still has several limitations preventing worldwide implementation -particularly in developing countries- such as high costs, which represents a life-long burden for patients and public health systems. In view of this it remains a necessity to develop prophylactic therapies capable of generating high levels of protective immunity at the time of virus contact with the host. A multi-epitopic vaccine approach offers multiple advantages over conventional immunogens, such as the amount of protein or nucleic acid required to generate protection is lower, the immune response can be directed towards conserved or normally subdominant epitopes, and facilitates the design of multi-clade spanning immunogens. Recently, Virus Like Particles (VLP) have attracted special interest to be adapted as antigen delivery and presentation systems due to their capability to stimulate both cellular and humoral responses, additionally, its three-dimensional structure allows for the presentation of conformational epitopes.

In the present study, two chimeric proteins based on the VP2 structural subunit of the B19 human parvovirus carrier system were designed, coupled to different HIV epitopes identified as binding sites for potent broadly neutralizing antibodies (bnAbs), cytotoxic lymphocytes and T-helper cells. The chimeric proteins, named MHIV-A and MHIV-B were designed and codon optimized for its recombinant expression in E. coli and chemically synthetized for its downstream cloning in the pET22b+ expression system. The chimeras were expressed in high concentrations (up to 65mg recombinant protein/ culture L), and it was determined that the inclusion bodies displayed an altered solubility pattern in comparison to wild-type (WT) VP2. Finally, both chimeras were

refolded and successfully assembled in vitro into VLPs using different molar ratios, such as 1:3 (chimera: WT VP2), and interestingly, we were able to assemble full capsids (size 16 -25 nm) using only chimeric protein, which had not been reported to date with the VP2 antigen delivery system.

Targeting of rotavirus VP6 to langerin induces a strong protective immune response against the infection in mice

Oscar Badillo-Godinez[1], Gerardo Espino-Solis[2], Delia López-Guerrero[3], Juan Villegas-Garcia[3], Yunuen Acevedo-Betancur[3], Francisco Velásquez-Hernández[4], Gerard Zurawski[5], Sandra Zurawski[5], lourdes Gutiérrez-Xicotencatl[1], Fernando Esquivel-Guadarrama[3]

[1]Instituto Nacional de Salud Pública, Cuernavaca, Mexico
[2]Autonomous University of Chihuahua, Chihuahua, Mexico
[3]Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[4]Benito Juárez Autonomous University of Oaxaca, Oaxaca, Mexico
[5]Baylor Health Care System, Dallas, United States
badillo.oscar@gmail.com; espinogp@gmail.com; vannylpz@yahoo.com; jc_villegas_g@hotmail.com; yunuab23@hotmail.com; velasquez3012@outlook.com; gerardz@baylorhealth.edu; sandraz@baylorhealth.edu; mlxico@insp.mx; fernando.esquivel@uaem.mx

Keywords: Dendritic Cells, Rotavirus, targeting, langerin, VP6

Rotavirus (RV) is the primary etiologic agent of severe gastroenteritis in human infants and neonates of different species, mainly in developing countries. Although two vaccines based on attenuated RV have been licensed to be applied around the world, in low-income countries are not so effective and the precise mechanisms of the protection induced are not clearly established. In this way, it is important to develop new generation vaccines that induce long lasting heterotypic immunity.

The protein VP6 constitutes the middle layer of RV and is the most conserved, abundant and immunogenic protein of the virion. Thus, VP6 is considered a potential vaccine antigen for the generation of a heterotypic protection against the infection. In fact, it has been shown in the mouse model that VP6 administered intranasally (i.n.) can induce a very efficient T cell dependent protective response against the infection at the intestinal mucosa level. However, the i.n. route of vaccination still is not feasible in humans for potential adverse effects, therefore, the parenteral route has been tested but very low levels of protection have been observed. This may be the result of the poor induction of the mucosal homing receptors α4β7 y CCR9 on the T cells induced in peripheral lymphoid organs. Nevertheless in previous study of our group (Badillo O. et al., 2015), it was demonstrated that when VP6 is conjugated to a monoclonal antibody (mAb) against DEC-205, manly present on dendritic cells (DCs) and inoculated subcutaneously (s.c.), in the presence of an adyuvant, a protection against the infection

(20-40 %) was observed. Indicating that if targeted to DCs VP6 can induce a protective immune response at the intestinal mucosal level, even when inoculated parenterally. This protection was independent of intestinal IgA antibodies and correlated to a Th1-type response.

The aim of the present work was to evaluate whether VP6 could be targeted to other C-type lectin membrane protein present in the DCs, such as Langerin, and induce a protective immune response against the infection when administered parenterally. The platform Dockerin-Cohesin from the bacteria cellulosome (Carvalho AL et al 2003) was used to generate conjugates of a rat anti-mouse Langerin mAb (2G3) and VP6. HEK-293 cells were co-transfected with a plasmid codifying for the heavy chain of the rat anti-mouse Langerine mAb fused to Dockerin and a plasmid codifying for the light chain, and purified from the supernatant. On the other hand, HEK-293 cells were transfected with VP6 fused with Cohesin, and the recombinant protein purified from the supernatant and conjugated to the recombinant mAb. The conjugates retained their capacity to recognize Langerin on bone marrow-derived DCs (Figure 1). BALB/c mice were inoculated s.c. with the amount of the conjugate equivalent to 5 µg of VP6, as controls, a group of mice was inoculated with a conjugate of a control isotype mAb and VP6 or with 5 µg of VP6 alone. All the immunization were performed in the presence of 50 µg of Poly I:C and repeated 21 d after. Sera was obtained before each immunization. Two weeks after the last immunization, mice were either challenge orally with the murine RV EDIM, to analyzed viral load and anti-RV IgA antibodies in feces, or sacrificed to evaluate the production of cytokines in vitro by anti-VP6 T-helper (Th) cells from spleen and the mesenteric lymph nodes (MLN). It was found that the αLangerine-Dockerine-Cohesine-VP6 conjugates induced high levels of IgG anti-VP6 antibodies compared to the controls. Also, the mice immunized with the αLangerine-Dockerine-Cohesine-VP6 conjugates showed up to 57 % protection against the infection, and in comparison, the controls did not show any significant protection (Figure 2 A, B). This protection was independent of intestinal anti-RV IgA antibodies as all the groups showed similar levels (Figure 2C). Anti-VP6 Th cells from

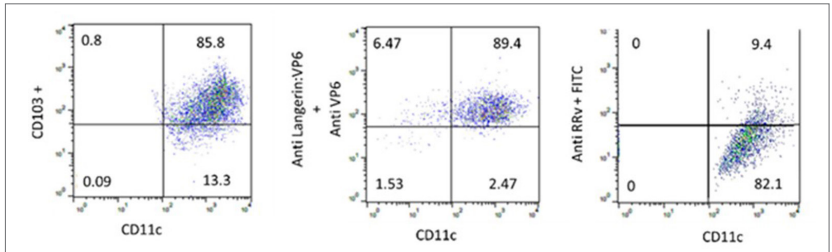


FIGURE 1

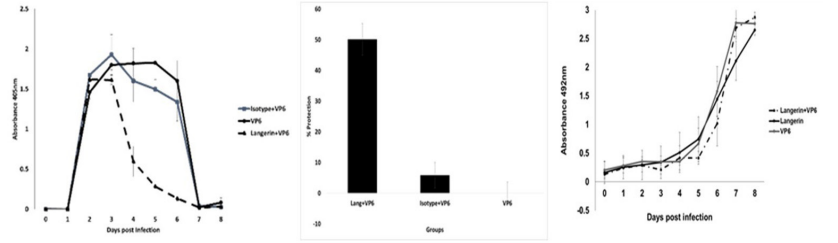


FIGURE 2

spleen and MLN showed a predominant pro-inflammatory profile since they produced higher levels of IFN-γ and TNF-α than IL-4 and IL-10. Finally, to demonstrate that the protection was dependent on Th cells, before and during the RV challenge mice were depleted of this cell population inoculating intraperitoneally (i.p.) purified rat anti-mouse CD4 mAb (GK 1.5). It was, found that depletion of the Th cell population completely abrogated the protection against the infection. This results show that, as with DEC-205, VP6 targeted to Langerin on the DCs can induce a strong protection against the infection at the intestinal mucosa when administered parenterally, and that this protections depends on Th1-type cells. These results could contribute in the future for the design of a new generation RV vaccine.

REFERENCES

Badillo-Godinez O, Gutierrez-Xicotencatl L, Plett-Torres T, Pedroza-Saavedra A, Gonzalez-Jaimes A, Chihu-Amparan L, et al. Targeting of rotavirus VP6 to DEC- 205 induces protection against the infection in mice. Vaccine 2015;33:4228–37.

Carvalho AL, Dias FM, Prates JA, Nagy T, Gilbert HJ, Davies GJ, Ferreira LM, Romao MJ, Fontes CM. Cellulosome assembly revealed by the crystal structure of the cohesin-dockerin complex. Proc Natl Acad Sci U S A. 2003; 100:13809–13814

Evaluation of the memory immune response induced by the novel pertussis formulation based on outer membrane vesicles

Maria Eugenia Zurita[1], M M Wilk[2], Alicja Misiak[2], Kingston Mills[2], Daniela Hozbor[1]

[1]CONICET Instituto de Biotecnologia y Biologia Molecular (IBBM), La Plata, Argentina
[2]Trinity College, Dublin, Dublin, Ireland
eugeniazurita@gmail.com; WILKM@tcd.ie; MISIAKA@tcd.ie; millsk@tcd.ie; hozbor.daniela@gmail.com

Keywords: Bordetella pertussis, Vaccines, Whooping Cough, resident memory T cells, Outer membrane vesicles (OMVs), Memory immune responses

Pertussis is a respiratory disease caused by the Gram-negative bacteria Bordetella pertussis that despite being immune preventable remains one of the leading causes of death in children under one year. In the last two decades, the incidence of this acute infectious respiratory disease has increased in many countries; with around 16 million cases occurring per year associated with approximately 200,000 deaths [1]. Most of those cases have been reported in developing countries; although in recent years large outbreaks have also been detected in developed countries, even in those with high vaccination rates [1-3]. This epidemic detected in different countries has moved the scientific community and health professionals to seek an understanding of this alarming new situation, to identify the causes [2, 4, 5], and review and implement new strategies for the control of pertussis [6]. Several factors apparently contribute to this pertussis-case increase, probably some occurring at a different weight depending on the country and the population considered. Nevertheless, a consensus exists in identifying, as part of the causes of the epidemic, several factors related to the vaccines currently in use and the vaccination — e.g. suboptimal coverage of the three primary doses, noncompliance with vaccination-schedule timing (delayed vaccination) [7, 8], the waning of vaccination-conferred immunity [9-11], and the circulation of a resistant bacterial causative-agent population resulting from the selection pressure exerted by mass vaccination [5]. Currently two types of vaccines against pertussis are in use: the whole-cell vaccines (wP) constituted by a suspension of detoxified heat-killed bacteria and acellular vaccines (aP) consisting of purified B. pertussis immunogens. wP was the first developed against the disease. To improve the control strategies to the disease we have developed a new vaccine candidate based on outer membrane vesicles (OMVs) capable of inducing a longer-term robust immune response [12-16].

Recently it has been shown that the natural infection is capable of inducing a systemic immune response with central memory T-lymphocytes (TCM) and local memory T cells with resident memory lymphocytes (TRM). In the frame of the vaccination with whole cell vaccine (wP) both populations are induced, while with the acellular vaccine (aP) the TRM population is absent. These results correlate with the reports that indicate that the duration of the immunity conferred by aP is shorter than with wP. In order to evaluate the ability of our vaccine candidate to induce TRM lymphocytes in the lung, in vivo assays were performed in which the systemic and local response was evaluated, in comparison with the wP vaccine. We found that in the lungs of the group of animals immunized with OMVs, as occurred in wP immunized animals, TRM lymphocytes are induced (14.8 × 10 4 cells/lungs in both cases). These lymphocyte population is capable of secreting IFN-γ in response to specific stimuli: 8.49ng/ml for OMVs immunized animals and 7.84ng/ml for wP immunized animals. When we analyzed the systemic immune response, we found that both formulations present a mixed profile with secretion of IFN-γ [OMVs: 179.55 ng / ml; wP: 114.29ng / ml] and IL-17 [OMVs: 1.84ng / ml; wP: 0.25ng / ml]. The results obtained indicate that immunization with the formulation based on OMVs is capable of inducing memory resident lung lymphocyte populations, which would explain the longer duration of the immunity induced by kind of formulation.

REFERENCES

1. Anon. Pertussis vaccines: WHO position paper. Releve epidemiologique hebdomadaire / Section d'hygiene du Secretariat de la Societe des Nations = Weekly epidemiological record / Health Section of the Secretariat of the League of Nations 2010; 85(40): 385-400.
2. He Q, Mertsola J. Factors contributing to pertussis resurgence. Future microbiology 2008; 3(3): 329-339.
3. Clark TA. Changing pertussis epidemiology: everything old is new again. The Journal of infectious diseases 2014; 209(7): 978-981.
4. Jackson DW, Rohani P. Perplexities of pertussis: recent global epidemiological trends and their potential causes. Epidemiology and infection 2014; 142(4): 672-684.
5. Bart MJ, et al. Global Population Structure and Evolution of Bordetella pertussis and Their Relationship with Vaccination. mBio 2014; 5(2).
6. Forsyth K, et al. Strategies to Decrease Pertussis Transmission to Infants. Pediatrics 2015.
7. Gentile A, et al. [Delayed vaccine schedule and missed opportunities for vaccination in children up to 24 months. A multicenter study]. Archivos argentinos de pediatria 2011; 109(3): 219-225.
8. Pesco P, et al. Mathematical modeling of delayed pertussis vaccination in infants. Vaccine 2015.
9. McGirr A, Fisman DN. Duration of Pertussis Immunity After DTaP Immunization: A Meta-analysis. Pediatrics 2015.
10. Klein NP, et al. Waning protection after fifth dose of acellular pertussis vaccine in children. The New England journal of medicine 2012; 367(11): 1012-1019.
11. Wendelboe AM, et al. Duration of immunity against pertussis after natural infection or vaccination. The Pediatric infectious disease journal 2005; 24(5 Suppl): S58-61.

12. Hozbor DF. Outer membrane vesicles: an attractive candidate for pertussis vaccines. Expert review of vaccines 2016: 1-4.
13. Asensio CJ, et al. Outer membrane vesicles obtained from Bordetella pertussis Tohama expressing the lipid A deacylase PagL as a novel acellular vaccine candidate. Vaccine 2011; 29(8): 1649-1656.
14. Roberts R, et al. Outer membrane vesicles as acellular vaccine against pertussis. Vaccine 2008; 26(36): 4639-4646.
15. Bottero D, et al. Characterization of the immune response induced by pertussis OMVs-based vaccine. Vaccine 2016; 34(28): 3303-3309.
16. Gaillard ME, et al. Acellular pertussis vaccine based on outer membrane vesicles capable of conferring both long-lasting immunity and protection against different strain genotypes. Vaccine 2014; 32(8): 931-937

Why are moonlighting proteins so abundant in pathogen microorganisms virulence and so scarce as protective subunit vaccines?

Luis Franco Serrano[1], Gabriela Ferragut[2], Josep Perez-Pons[1], Angel Mozo-Villarias[1], Jaume Piñol[1], Isaac Amela[1], Enrique Querol[1], Juan Cedano[2]

[1]Universitat Autònoma de Barcelona, Barcelona, Spain
[2]University of the Republic, Montevideo, Uruguay
francoserrano.luis@gmail.com; gferragu@fq.edu.uy; josepantoni.perez@uab.cat; angmovi@gmail.com; jaume.pinyol@uab.cat; iamela@bioinf.uab.cat; Enric.Querol@uab.cat; jcedano@unorte.edu.uy

Keywords: Immunization, Vaccines, bioinformatics, pathogens, Moonlighting Proteins

Moonlighting or multitasking is the capability of some proteins to execute two or more biochemical functions. Most human pathogen microorganisms use moonlighting proteins as virulence factors for the colonisation of the host and subvert its immune system. We have designed a database of moonlighting proteins (http://wallace.uab.es/multitaskII) and 25% of its 694 proteins correspond to proteins involved in human pathogen virulence. Mostly their canonical function is as enzyme of the central metabolism or key pathways, i.e., glycolysis, Krebs cycle, etc., and they are shared by different species of pathogen microorganisms. They are secreted out of the pathogen cell but lack of signal peptide or other secretion motifs (as LPXTG, etc). In addition, most of them interact with plasminogen and other extracellular matrix proteins facilitating the colonization of the host tissues, complement system evasion and disrupting the immune response. All these facts require an explanation. We propose that as these ancestral and key metabolism proteins are evolutionally highly conserved in amino acid sequence and structure with the host orthologue counterparts. Thus, they likely will share epitopes with it, and the epitope mimicry with host proteins which could be cause of autoimmune diseases. Therefore, the host immune system would avoid eliciting a strong immune response to elude an autoimmune condition. This is key for recombinant subunit vaccine design because although can be antigenic, they would not elicit a protective immune response.

U-OMP19 from brucella abortus increases dmlt immunogenicity and improves protection against heat-labile Toxin (LT) oral challenge in vivo

Franco Luis Martinez, Mirta Lorena Coria, Laura Bruno, Celeste Victoria Pueblas Castro, Juliana Cassataro

Instituto de Investigaciones Biotecnológicas (IIB-INTECH), Chascomús, Argentina
franco3300@gmail.com; lorecor@gmail.com; inmuno24@gmail.com; celes1906@hotmail.com; jucassataro@yahoo.com.ar

Keywords: Adjuvants, Immunologic, Vaccines, ETEC, Oral delivery formulation, U-Omp19

Enterotoxigenic E. coli (ETEC) is the most common cause of bacterial diarrhea in developing countries. Both naturally acquired infection and oral-mucosal vaccination against heat-labile toxin (LT), colonization factors or adhesins can induce protective immunity. So, LT is being used as oral adjuvant/antigen (Ag) in mice. Since its toxicity limits its practical use in humans, a double mutant of LT (dmLT) which is less toxic and retains its adjuvant properties is under clinical trial.

U-Omp19 is a protease inhibitor from Brucella spp. with immunoestimulatory properties. We propose to use U-Omp19 as platform to deliver antigens (Ags) in oral formulations against infectious diseases. Previously, it has been shown that this protein can protect co-administered Ags from digestion and it can also trigger and direct the type of immune responses against the Ag. In this work our aim was to investigate the effect of U-Omp19 co-delivery on dmLT immunogenicity and protective efficacy in vivo. To this end inbred BALB/c or outbred CD1 mice were orally immunized, according to different protocols, with i) saline ii) dmLT or iii) dmLT+U-Omp19. Three doses of dmLT were studied alone or plus U-Omp19: 25μg, 12,5μg and 2,5μg. Fecal and serum α-LT antibodies (Abs) were evaluated by ELISA every week and after last immunization mice were challenged orally with LT enterotoxin (patent mouse gut assay). Results obtained indicated that co-delivery of U-Omp19 increased ($P<0.05$) mucosal and systemic IgA and IgG α-LT Abs and avidity of systemic IgA α-LT. Moreover, U-Omp19 when co-delivered with dmLT induced significant protection ($P<0.05$) against oral challenge with LT in BALB/c and CD1 mouse model, while dmLT alone did not. All together our results indicated that U-Omp19 can help to increase dmLT immunogenicity and produce neutralizing antibodies against LT in vivo. So, U-Omp19 would be a good candidate to be included in dmLT vaccine formulations against ETEC.

Evaluation of the immunogenicity of Zika virus envelope protein domain III encapsulated in nanoparticles

Karen Bernal-Siria, Julio García-Cordero, Mayra Montecillo-Aguado, Goldie Oza, Leticia Cedillo-Barron

Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico

karen.bernalsiria@gmail.com; leebeydengue@yahoo.com.mx; mayramontecillo@hotmail.es; goldieoza@gmail.com; lcedillo@cinvestav.mx

Keywords: Vaccines, Chitosan nanoparticles, PLGA nanoparticles, Envelope protein, Zika

Background: After Zika virus (ZIKV) was associated with teratogenic symptoms in fetuses and with Guillaine-Barré syndrome(GBS), the World Health Organization declared Zika infection as a global public health emergency in 2016(1). Although there is still no approved vaccine for prevent infection.

One of the most important obstacle for the development of the vaccine is the high similarity between the sequences of ZIKV and Dengue Virus (DENV), this similarity conditioned the antibody-dependent enhancement (ADE) described for various dengue serotypes and by which antibodies against Zika in a population free of DENV could also facilitate the severe DENV clinical forms. On the other hand, vaccination could increase the chances of developing or worsening GBS, because there is the hypothesis that the mechanism by which the disease is triggered is molecular mimicry(2), for that reason the use of inactivated virus and subviral particles (SVP) need to be avoided until discard that mechanism.

Therefore, it is necessary to work on the development of specific vaccines to minimize both, the risk of severe DENV infections and the development of GBS conditioned by ZIKV vaccines. One of the strategies to achieve specific vaccines is the design of peptide subunit vaccines. It has been demonstrated that immunization of mice with DIII is able to generate a specific and neutralizing antibody response against ZIKV(3). However, for the use of a soluble antigen it is essential to include adjuvant molecules whit the potential to increase the immune response. Recently it has been directed attention to the use of nanoparticles as administration vehicles for vaccines, these allow the prolonged release of antigens, protect their integrity until their endocytosis by the cells, and can serve as adjuvants. In addition, it allows the cross-presentation of antigens, which is especially important for the generation of cellular responses against viral infections(4).

The aim of this study was to evaluate the immunogenicity of Zika virus DIII in a mice model evaluating the adjuvant effect of PLGA nanoparticles and chitosan.

Methodology: The Zika virus was obtained from an isolate from Oaxaca that correspond to Asian lineage. C6/36 cells was infected with this virus to get viral RNA, and RT-PCR was performed to obtain cDNA. The DIII of the envelope protein of Zika virus was amplified by PCR, the amplified was inserted into the vector pProEX HT-B, and BL-21 bacteria were transformed with this construct, to obtain a protein with a exa-histidine tag. Subsequently the protein was produced on a large scale, the inclusion bodies were isolated and the protein was purified by preparative gels. The purity of the protein was verified by SDS-PAGE with Coomassie and identified by western blot. Next, PLGA and chitosan nanoparticles were synthesized with and without the encapsulated antigen and 6 groups of Balb/c mice were immunized with 25 μg/ml of DIII mixed with: PBS, aluminum, PLGA nanoparticles, chitosan nanoparticles, PLGA nanoparticles containing the encapsulated antigen, or chitosan nanoparticles with encapsulated antigen. These mice were injected at day 0 and boosted on day 21, and serum was collected at day 21 and 42. Finally, the humoral immune response was evaluated through the analysis of antibodies against DIII by ELISA.

Results: The domain III sequence was amplified from cDNA and cloned into the zDI-II-pProEX HT-B vector in BL-21 cells. The resulting colonies were analized with Xba I and Xho I enzymes to release the DIII sequence, demonstrating the transformation of three colonies.

The expression kinetics was analyzed by SDS-PAGE after induction with IPTG, obtaining a maximum expression of the protein from 3 hours post-induction. Domain III purification was demonstrated by SDS-PAGE with Coomasie and it was identified by Western Blot using an anti-histidine antibody.

No significant differences were observed in antibody response produced by domain III between the formulations containing PLGA or chitosan nanoparticles and those containing aluminum as adjuvant. Likewise, no differences were observed between the level of antibodies in mice immunized with the antigen mixed and encapsulated and those immunized with the antigen only mixed with nanoparticles

Conclusion: Our data show that domain III inoculated with PLGA or Chitosan nanoparticles is capable to generate a humoral immune response similar to that obtained with aluminum. However, it is necessary to characterize and improve the encapsulation process in order to obtain better responses with the use of these nanoparticles for immunization against DIII, as well as to analyze if neutralizing antibodies against Zika virus are present in serum of immunized mice.

REFERENCES

1. WHO. Fifth meeting of the Emergency Committee under the International Health Regulations (2005) regarding microcephaly, other neurological disorders and Zika virus. http://www.who.int/mediacentre/news/statements/2016/zika-fifth-ec/en/. Published 2016.
2. Anaya J-M, Ramirez-Santana C, Salgado-Castaneda I, Chang C, Ansari A, Gershwin ME. Zika virus and neurologic autoimmunity: the putative role of gangliosides. BMC Med. 2016;14(1):49. doi:10.1186/s12916-016-0601-y.
3. Yang M, Dent M, Lai H, Sun H, Chen Q. Immunization of Zika virus envelope protein domain III induces specific and neutralizing immune responses against Zika virus. Vaccine. 2017;35(33):4287-4294. doi:10.1016/j.vaccine.2017.04.052.
4. Oyewumi MO, Kumar A, Cui Z. Nano-microparticles as immune adjuvants: correlating particle sizes and the resultant immune responses. Expert Rev Vaccines. 2011;9(9):1095-1107. doi:10.1586/erv.10.89. Nano-microparticles.

Quillaja brasiliensis leaf saponins and their nanoparticles are strong inducers of early immune responses

Mariana Rivera-Patron[1], Samuel Paulo Cibulski[2], Gustavo Mourglia-Ettlin[3], Cecilia Casaravilla[3], Anna Carolina Yendo[2], Arthur Germano Fett-Neto[2], Jose Alejandro Chabalgoity[1], María Moreno[1], Paulo Michel Roehe[2], Fernando Silveira[1]

[1]Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[2]Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
[3]Facultad de Química, Universidad de la República, Montevideo, Uruguay
marianariverap@gmail.com; spcibulski@gmail.com; gmourglia@higiene.edu.uy; ccasarav@higiene.edu.uy; anna.yendo@gmail.com; fettneto@gmail.com; jachabal@higiene.edu.uy; mmoreno@higiene.edu.uy; proehe@gmail.com; fernandosilveiragonzalez@gmail.com

Keywords: Adjuvants, Immunologic, Nanoparticles, Saponins, early immune response, Quillaja brasiliensis, QB-90, Immune related genes

Background: Adjuvants have been used in veterinary and human vaccines for almost a century improving vaccine immunogenicity. Triterpenoid saponins, such as Quil A®, extracted from the barks of Quillaja saponaria Molina, have been widely used as adjuvants for many years in animal health vaccines. Although these compounds are able to trigger strong immune responses, their use in human vaccines has been relatively restricted due to undesirable side effects, such as local reactions, haemolytic activity and systemic toxicity. Throughout the last decade our group has been studying the adjuvant properties of saponins extracted from the leaves of Quillaja brasiliensis (A. St.-Hil. et Tul.) Mart., a native tree from southern Brazil and Uruguay. These saponins constitute a sustainable alternative source of saponins and were found to share structural and biological activities with Q. saponaria but inducing lower levels of toxicity than Quil A®, and yet strongly potentiating humoral and cellular immune responses to viral antigens. In order to reduce the undesirable side effects, saponins have been included in different colloidal preparations such as the immunostimulating complexes (ISCOMs). These 40 nm cage-liked self-assembled structures combine Quil A®, cholesterol, phospholipids and antigen. A similar preparation, ISCOMATRIX, does not include the antigen. As an alternative, our group has formulated for the first time ISCOMs replacing Quil A® by the Q. brasiliensis saponin fraction QB-90 (IQB-90). This formulation with reduced haemolytic activity promoted a strong humoral

and cellular immune response. When intranasally delivered, IQB-90 elicited serum IgG and IgG1, and mucosal IgA responses at distal sites, even with low antigen doses. Despite the fact that saponin-based adjuvants (SBAs) have been studied for nearly 30 years, their mechanisms of action are still not fully elucidated. Improving the understanding of such mechanisms, and in particular of SBAs derived from Q. brasiliensis, is highly relevant, as they constitute a readily renewable alternative source of saponins with reduced toxicity compared to Q. saponaria bark-derived saponins. In this study we provide a deeper insight into the immune stimulatory properties of QB-90 and ISCOMATRIX nanoparticles based on this fraction (IMXQB-90).

Results: Firstly, we showed that QB-90 promoted an "immunocompetent environment" when used as a viral vaccine adjuvant (when subcutaneously inoculated in the hind limbs twice at two weeks interval). Secondly, the ability of QB-90 and IMXQB-90 to stimulate leukocyte recruitment in draining lymph nodes (dLNs) and spleen 24 and 48 hours post-inoculation (hpi) was evaluated. The findings revealed that both formulations significantly promoted recruitment of neutrophils, dendritic cells (DCs), NK, B and T cells (when subcutaneously injected at the base of the tail). Subsequently, a differential production of IL-1β by bone-marrow derived dendritic cells (BMDCs) was detected following stimulation with QB-90 or IMXQB-90, involving Caspase-1/11 and MyD88 pathways, implying canonical inflammasome activation. To better characterize the cytokine profile induced by QB-90 and IMXQB-90 in mice, cytokines from sera obtained at 24 and 48 hpi were analyzed using a Th1/Th2/Th17 Cytokine bead array (BD). IFN-γ, and TNF-α were detectable in sera obtained at 24 hpi. The cytokine profile in either QB-90 or IMXQB-90-sensitized mice presented a similar increase in IFN-γ levels. TNF-α showed a significant increase only in QB-90-inoculated mice. None of the assessed cytokines were detected at 48 hpi. Finally, the effect of QB-90 and IMXQB-90 on the expression of immune-related genes was examined in mouse dLNs. Both formulations induced a change in the expression of genes coding for cytokines, chemokines and co-stimulatory molecules, many of which were shown to be up-regulated.

Conclusions: Herein we demonstrate that QB-90 induced an antigen-independent and transient immunocompetent environment. Early cellular responses were observed for both QB-90 and IMXQB-90, at local (dLN) and systemic (spleen) levels. Interestingly, IMXQB-90 showed a less intense systemic pro-inflammatory response, whilst still being able to activate by itself - at least in vitro - the inflammasome through Caspase-1/11 and MyD88 pathways. Moreover, in terms of number of immune related genes which changed expression, IMXQB-90 also seemed to induce a less intense inflammation in the dLN. These findings provide important insights into the molecular and cellular

mechanisms involved in SBAs, particularly regarding saponins of Q. brasiliensis and respective nanoparticles.

Overall, our results reinforce the postulation of using saponins from Q. brasiliensis - or their nanoformulations - to induce potent adjuvant activities in vaccines.

Challenging the vaccine adjuvant´s Gaston definition

Oliver Pérez[1], Laura María Reyes Díaz[2], Alexander Batista Duharte[3], Raúl Ramos Pupo[2], Rocmira Pérez Nicado[4], Yusnaby Borrego[2], Dainerys Aleida Pérez Lastre[5], Roberto Ricardo Grau[6]

[1]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón y ELAM, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba

[2]Instituto de Ciencias Básicas y Preclínicas "Victoria de Girón", Universidad de Ciencias Médicas de la Habana, La Habana, Cuba

[3]Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, Araraquara, Brazil

[4]Instituto Finlay, La Habana, Cuba

[5]Clinica Curitiba, Curitiba, Brazil

[6]Universidad de Rosario, Rosario, Argentina

oliverperez@giron.sld.cu; lreyes@giron.sld.cu; batistaduhartea@gmail.com; raulramos@infomed.sld.cu; rpnicado@finlay.sld.cu; yborrego@giron.sld.cu; dperezlastre@gmail.com; robertograu.conicet@gmail.com

Keywords: Vaccines, innate immunity, adjuvants, cross-protection, trained immunity

Introduction: The father of adjuvants, Ramón Gaston in 1925, defined them as a substance used in combination with a specific antigen that produces a more robust immune response than the antigen alone. So, adjuvant is a substance that: act unspecifically over innate immune response; increase the specific acquire immune response against a particular antigen, and make possible the development of subunit vaccines. They can reduce the antigen concentration permitting having more vaccines. They immunopolarize inducing: bactericidal and opsonic (coming from a Th1 pattern) or anaphylotoxic antibodies (IgE coming from a Th2 pattern); cytotoxic T lymphocytes (CTL, TCD8+ or TCD4+); neutralizing antibodies (coming from any pattern, meaning not polarization); and CTL memory response which only occur when TCD8+ received help from Th1. They increase the speed, intensity, affinity, and duration of the immune response. They could permit to functioning in extremes ages (new born and senescence) and in immunosuppresses subjects. They could solve the antigen immunodominancy in combined vaccines and could permit to be effective by different routes, particularly by mucosal one. Multiple adjuvant classification exists. Nevertheless, Singh and O'Hagan simplify them to Immunopotenciator and Delivery System1, which are not excluding and Pérez et al. added the Immunopolarizador to focalize the vaccine formulation over the required protective immune response2. Lastly, Schijns et al. and Pérez et al. classified them as facilitators of the immune signal3,4. Therefore, advances in the understanding of the mechanisms of innate immune response permits to overcome the empirical adjuvant research to the rational one. Gaston´s definition is driven to

the addition of exogenous adjuvants as most vaccinologist think. In addition, innate immune response acting immediately and with non-clonal specificity induces most protection than acquire immune response which can be effective over different pathogens5, mainly inducing cross-protective responses. This fact is undervaluing in the vaccinology field which was mainly focus in looking for new protective antigens. Lastly, the development of adjuvant takes several year and need to be tested with each new vaccine candidate formulation. Their preclinical evaluation is mainly conducted in isogenic mice, with limited number of animals, and most of the time the results could not be extrapolated to human.

Aims: To identified the challenge of adjuvant´s Gaston definition, to review some old and new aspects of antibody´s cross-reactive responses, and to test the hypothesis of cross-protection at animal production scale.

Results: We consider four challenges to Gaston´s definition. First, adjuvants are mostly self-one. Substances with adjuvant properties are mainly part of complex antigen formulations like occur with live attenuated and inactivated vaccines. Consequently, the majority of vaccines contain adjuvants and when we deprived of them using purified antigen in order to reduce reactogenicity, then, we need to added exogenous adjuvants. Combination of adjuvants is the hallmark, but Alum is prevalent in both (self and exogenous) adjuvantated vaccines. Only few plain polysaccharide vaccines are not using exogenous adjuvants. Consequently, they did not induce memory response which was overcome by covalent conjugation. In addition, we demonstrated that addition of a potent adjuvant non-covalently added also overcome the thymus-independence of polysaccharide vaccine, inducing Th1 and memory immune response6. Second, antigen specificity is the hallmark of acquire immune response. Nevertheless, most IgE induced against helminthic antigens or allergens are unspecific. In addition, IgA can be induced as thymus-dependent and -independent one7,8. Third, the mucosal route of immunization requires that adjuvant make additional task than parenteral one. They need to be adhesive, disrupt or traverse the mucosa layer, open tick junction, etc. Four, adjuvant alone (without any antigen) could protect cross-reactively against different pathogens. This is in agreement with the trained immunity of innate response9. This is a new adjuvant paradigm and a challenge to vaccinologist. So, why not protects using only adjuvants? In order to advance in these aspects we review some old and new aspects of antibody´s cross-reactive responses. Firstly, what is cross-reaction in immunology? Cross-reactivity measures the extent to which different antigens appear similar to the immune system, contrary to specificity which measures the degree to which the immune system differentiates between different antigens, one of the hallmarks of acquired immune response. Some cross-reactive antigens are induced by

protein components present in different organisms or allergens, probably reflecting the phylogenetic relations between them10; but other are cross-reactive carbohydrate determinants11. Protein specificity immune response is normally of high affinity and it is against the same (primary to quaternary) structures. The latter has lower affinity and it is probably against similar but not the same polysaccharide structure12. Cross-reactive immune response is a real problem for diagnostic purpose; but it was conserved during evolution to cross-protection against different aggressions. IgA is more cross-reactive than IgG meaning recognition of the same or similar antigens in different aggressions particularly from microbiota13. Therefore, they could be stimulated immediately by B1 cells. Consequently, they could stimuli cross-protective immune response at innate immune arm. Most of the infections and initial tumors are controlled by the immune surveillance i.e. innate immune response. Nevertheless, using cross-reactive antigens for vaccine development was not addressed and this is one of the adjuvant´s challenge mentioned above. To test the hypothesis of cross-protection at animal production scale we used gram+ Basillus subtillis probiotic to protect Claria garepinus fish. Mothers were immunized with only one dose before extraction of ova and their fertilization was measured indirectly by larvae new born. Alevins and young adults were challenged for 5 days one a day with B. subtillis spores. The survival and weigh increasing were measured. The significant increase of fertilization ova, survival of alevins and weigh increased were obtained.

Conclusions: Four changes need to be considering in adjuvant definition. Most antibodies induce during helminthiasis and allergy diseases and against microbiota are unspecific pointed out that cross-reactive responses are induce. Cross-protection at animal production scale is inducing using probiotic as adjuvant protecting and increasing the weight of fishes.

REFERENCES

1. Singh M & O'Hagan DT. Pharm. Res. 2002; 19(6):715-728.
2. Pérez O, et al. PharmacologyOnline, 2006; 3:762-64.
3. Schijns V. et al. Cytotherapy, 2014; 16:427e439.
4. Pérez O et al., Frontier Immunology, 2015; doi: 10.3389/Fimmu.2013.00407.
5. Pérez O e Veja Irma. Inmunología en el humano sano. Editora Ecimed 2017.
6. Romeu B, et al. Scand J Infec Dis, 2011; 43:809-813
7. Macpherson, A. J. et al. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science 2000; 288:2222-2226.
8. Cerutti A. The regulation of IgA class switching. Nature Reviews Immunology 2008; 8:421-434. doi:10.1038/nri2322).

9. Mihai G. Netea1, et al. Trained immunity: a program of innate immune memory in health and disease. Science. 2016; 22:352(6284). Doi:10.1126/science.aaf1098
10. Aalberse RC, Akkerdaas JH, R. van Ree. Cross-reactivity of IgE antibodies to allergens. Allergy 2001: 56:478-490.
11. Aalberse RC, Koshte V, Clemens JG. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 1981; 68:356-364.
12. Reyes Díaz LM, PhD thesis, 2018.
13. Bunker JJ, et al. Natural polyreactive IgA antibodies coat the intestinal microbiota. Science 2017; 20;358(6361). DOI: 10.1126/science.aan6619.

Prematurity and immune response to vaccine antigens

Yamilé Faguet Horta[1], Martha Leonor Paradoa Pérez[1], Carmen M Arenas[2], Irma Gudelia Vega García[1,3], Cira Virgen Rodríguez Pelier[1,4], Victoria Esther González Ramírez[1], Miriam SJB Lastre González[1], Oliver Pérez[1,3]

[1]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[2]Hospital Universitario Ginecobstétrico "Eusebio Hernández", Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[3]Escuela Latinoamericana de Medicina, La Habana, Cuba
[4]CNGEM, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
yamilefaget@infomed.sld.cu; mparadoa@infomed.sld.cu; carmen.arenas@infomed.sld.cu; irmav@giron.sld.cu; cira@cngen.sld.cu; victoria.goncalez@infomed.sld.cu; oliver.perez@infomed.sld.cu

Keywords: Infection, Premature Birth, Vaccines, low birth weight, Allergy disease

Premature is the newborn with less than 37 weeks of gestation1. High morbimortality occur on less than 28 weeks of gestation and a weight of less than 1000 g. Worldwide, prematurity is the leading cause of death in children under five years of age. Each year about 15 million babies are born before reaching term. South Asia and Sub-Saharan Africa account for half of the world's births, more than 60% of premature babies and more than 80% of the world's 1.1 million deaths due to complications from premature births. On the other hand, there is a dramatic survival gap for premature babies depending on where they are born. In this sense, half of babies born at 24 weeks survive in high-income countries. However, in low-income countries, half of babies born at 32 weeks still die due to lack of effective, feasible care and economic2. In Cuba, as gestational age and low birth weight (<2,500 g) correlate and this is taken as a marker for the follow-up of neonates. The rate of low birth weight was 5.1% in 20153. These children are not sufficiently developed to cope with the extrauterine environment without assistance. Their immaturity predisposes them to suffer an increased risk (3-10 times more) of infections. In addition, the underdevelopment of their immune system and their defenses severely compromises their ability to produce antibodies and mount protective and specific immune responses. Preterm children also tend to have lower levels of maternal IgG than a term infants, which leaves them vulnerable to the effects of many infectious agents4-6. The prevalence of infectious diseases is high in the pediatric age and it is highest in premature with lower respiratory infections and hospitalization; being among the most frequent causes of morbidity and mortality. All these are complicate by prematurity, as well as by the immaturity in the development

of your immune system7. There are many criteria about the immune response of the preterm child, especially in relation to vaccination. Studies with sufficient internal (scientific rigor) and external (generalization capacity) validities are scarce when it comes to specifying the efficacy, safety, reactogenicity, and immunogenicity of the different immunizations in the prematures8-10. Having no studies of these types in our country, we evaluate if prematurity affects the IgG anti tetanus toxin (TT) and hepatitis B surface antigen (HBsAg) and identify perinatal risk factors that affect this response.

Method: An epidemiological, cross-sectional, observational, and descriptive study was performed with children vaccinated with Heberpenta® vaccine (according to the national schedule of vaccination. Children of 2, 3, or 4 years born premature (45) or at term (30) were studied. ELISA was used to determine IgG anti-TT and HBsAg. Sadistic analysis were calculate using nonparametric Anova followed by Tukey using prism system and p<00,5 was use as significant value.

Results: The IgG and TT and anti-HBsAg antibodies evaluated in children of 3, 4, and 5 years old were similar (p>0,05) between those that were born premature or at term. Nevertheless, preterm with low birth weight (<2500 g) have significant (p<0,05) lesser IgG anti TT, but not anti HbsAg, than those born with normal weigh. This suggests that a reduced induction of anti TT IgG during vaccination schedule (3 doses finishing at 6 or 12 months in newborn coming form HB- or HB+ mothers, respectively) occur which persist for several years. This result is coinciding with other reports that evaluate retardation of the immune response against DPT vaccine11,12. Ninety tree and 100% of children against Tetanus (>0,1 IU/mL) and Hepatitis B (≥10 IU/L) were protected, respectively and these protections have being maintained for 4 years after the conclusion of the schedule. Only 6.8% of premature showed non-protective values of IgG anti TT (<0,1 IU/mL). In studies performed in our country, long-term protection levels were recorded in more than 65% of those born at term13, coinciding with what was observed in our work. At 5 years, 90% and at 7 years, 50-70% of those vaccinated still have anti-HBsAg antibodies14. In our study children, under 5 years old continue with high anti-HBsAg titers independent of were born premature or not. Thirty three and 3,3% (p=0,005) of those born premature and at term, respectively were hospitalized by infection and consequently, received antibiotic therapy. These affect also the acquisition of microbiota whcih plays a fundamental role on the induction, training, and function of the host immune system15,16,17. So, premature immune response induction was affected. In addition, a window of opportunities for the development of an adequate immune response was recently described at least in mice18,19. This is consistent with our significant (p<0,001) finding of 84.4% of allergic children in those born premature. Bacterial or viral infections can become more serious or even

lethal in premature children; influencing in many occasions, in the delay of the vaccination. Some newborns are deprived of precocious enteral nutrition, including breast milk, which deprives them of the passive immunity conferred against bacterial and viral pathogens. Infections with polysaccharide-mediated pathogenicity are especially severe in the first months of life. In the first two to three years, the response to these antigens is weak or nonexistent20. The most important neonatal factor that predisposes to infection is prematurity. Premature with gestational age less than 28 weeks, the relative risk of early neonatal death is higher than those born at term20. Together with the decrease in gestational age, the low weight at birth with <1500 g and those named "extremely low birth weight (<1000 g) are related to an increase in the perinatal mortality and morbidity rate21.

Conclusions: Prematurity and low birth weights predispose to infections, required antibiotic therapy and predispose to allergy in early life.

REFERENCES

1. González Merlo J. Parto pre término. Obstetricia. (2006). (5ta edición). Cap 38. España. in: http://books.google.es/books?
2. Goldenberg R, Culhane JF, Lams JD, Romero R (05-01-2008). Epidemiology and causes of preterm birth. Lancet 2008; 371:75-84.
3. Anuario Estadístico de Salud 2015. Ministerio de Salud Pública. Minsap-OPS-Unicef-FNUAP, La Habana.
4. Shankaran FA, Wright LL, Stevenson DK, Donovan EF, Ehrenkranz RA, Langer JC, Korones SB, Stoll BJ, Tyson JE, Bauer CR, Lemons JA, Oh W, Papile LA. Risk factors for early death among extremely low-birth-weight infants. Am J Obstet Gynecol 2012; 186:796-802.
5. Enciclopedia médica en español. Bebe prematuro. [https://medlineplus.gov/spanish/prematurebabies.html]. 2007. Consultado el 21.1.18.
6. Van de Berg JP, Westerbeek EA, Van der Klis FR, Bebers GA, Van Elburg RM. Transplacental transport of IgG antibodies to preterm infants: A review of the literature. Early Hum Dev. 2011; 87:67-72
7. Strunk T, Currie A, Richmond P, Simmer K, Burgner D. Innate immunity in human newborn infants: prematurity means more than immaturity. The Journal of Maternal-Fetal and Neonatal Medicine, 2011; 24(1):25-31.
8. Comité Asesor de Vacunas de la Asociación Española de Pediatría. Vacunación de niños prematuros. Manual de vacunas en línea de la AEP. Madrid: AEP; 2015; in: http://vacunasaep.org/documentos/manual/cap-10. Consultado el 12 21.1.18.
9. D'Angio CT, Heyne RJ, Duara S, Holmes LC, O'Shea TM, Wang H, et al. Immunogenicity of trivalent influenza vaccine in extremely low-birth-weight, premature versus term infants. Pediatr Infect Dis J. 2011; 30:570-574.
10. Esposito S, Fumagalli M, Principi N. Immunogenicity, safety and tolerability of vaccinations in premature infants. Expert Rev Vaccines. 2012; 11:1199-1209.
11. Woestenberg PJ, van Lier A, van der Maas NA, Drijfhout IH, Oomen PJ, de Melker HE. Delayed start of diphtheria, tetanus, acellular pertussis and inactivated polio vaccination in preterm and low birth weight infants in the Netherland. Pediatr Infect Dis J. 2014; 33:190-8.

12. Omeñaca F, Merino JM, Tejedor JC, Constantopoulos A, Papaevangelou V, Kafetzis D, et al. Immunization of preterm infants with 10-valent pneumococcal conjugate vaccine. Pediatrics. 2011; 128:e290-8.
13. Ochoa Azze RF. 2009. Inmunoepidemiología y Estrategias de Vacunación, 2da ed. Finlay Ediciones, Ciudad de la Habana.
14. Inmunización en prematuros: generalidades. Medwave. Revista Biomédicas. 2015 in http://www.medwave. Consultado el 12 21.1.18.
15. Belkaid Y and Hand T. Role of the Microbiota in Immunity and inflammation. Cell. 2014; 27,157(1):121-141. doi:10.1016/j.cell.2014.03.011.
16. Buffie CG and Pamer EG. Microbiota-mediated colonization resistance against intestinal Pathogens. Nat Rev Immunol. 2013; 13(11):790-801. doi:10.1038/nri3535
17. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nature reviews Immunology. 2013; 13:790-801.
18. Gomez de Agüero M, et al. The maternal microbiota drives early postnatal innate immune development. Science 2016;351(6279):1296-1302.
19. Gensollen T, et al. How colonization by microbiota in early life shapes the immune system. Science 2016; 352(6285):539-543.
20. Kiechl-Kohlendorfer U, Ralser E, Pupp Peglow U, Reiter G, Trawoger R. Adverse neurodevelopmental outcome in preterm infants: risk factor profiles for different gestational ages. Acta Pediatr 2013; 98:792-796.
21. Martius JA, Steck T, Oehler MK, Wulf KH. Risk factors associated with preterm (<37+0 weeks) and early preterm birth (<32+0 weeks): univariate and multivariate analysis of 106 345 singleton births from the 2004 statewide perinatal survey of Bavaria. Eur J Obstet Gynecol Reprod Biol 2008; 80(2):183-9.

Protection induced by intradermal immunization with Salmonella Typhi porins correlate with the induction of memory T cells and dendritic cell responses in skin and draining lymph node

Luis Alberto Ontiveros-Padilla[1], Alberto García-Lozano[1], Rodolfo Pastelin-palacios[2], Lourdes Arriaga[1], Armando Isibasi[1], Laura Bonifaz[1], Constantino López-Macías[1]

[1]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
luisalberto.ontiveros.p@gmail.com; jb_justdoit@hotmail.com; rodolfop@unam.mx; landapi@hotmail.com; isibasi@prodigy.net.mx; labonifaz@yahoo.com; constantino@sminmunologia.mx

Keywords: Porins, Salmonella typhi, Skin, protection, memory T cells, dendritic cell subsets

Salmonella enterica serovar Typhi (S. Typhi) porins are potent inducers of the protective immune response in mice and humans. Intraperitoneal immunization with porins induce protection against the challenge with 500 lethal dose of S. Typhi, and IFN-gamma specific T cells and life-lasting bactericidal antibody responses in mice. Unusual immunogenicity of porins has been attributed to its capacity to activate innate immune response through TLR2 and TLR4 on dendritic cells (DCs). Due to the central role of DCs in the activation of protective and long-lasting immune response, we explored intradermal immunization of porins (which is a place rich in DCs subsets), in order to characterize the mechanism involved in the long-lasting protective immune responses induced by porins.

Intradermal immunization of porins induced the reduction of the bacterial burden in the spleen and liver of S. typhi challenged mice and the production of high specific antibody titres, similar to the observed in mice immunized intraperitoneally. In addition, we observed the induction of memory T cell response in draining lymphoid nodes (DLN) and in the immunized skin. Surprisingly, we found an increased number of memory T CD4+ cells in the skin but not in DLN, suggesting that the memory response in the immunization site is important to induce a systemic protection against this pathogen.

Finally, we studied different DCs subsets activation, two resident subsets (rDC) and four migratory subsets (miDC) in DLN and Langerhans cells activation phenotype in the skin. We observed an activated phenotype in Langerhans cells of the immunized skin at early times post-immunization and an increased number of the six DCs subsets

in DLN at 72h post-immunization. CD8alpha- DC subset was the dominant resident population in DLN, whereas CD11b- Langerin- was the larger migratory population, suggesting both DC subsets could be involved in the induction of the T cell response observed.

Taken together, our data shows that intradermal immunization of porins are able to induce systemic protection and activate T cell memory responses and different subsets of DCs which may be important in the induction of protective immune responses.

The Immunology in the university formation, research, and translational products in Cuba

Oliver Pérez[1,2], Irma Gudelia Vega García[1,2], Alina Josefina Alerm González[1,2], Carlos Villegas Valverde[2,3], Victoria Esther González Ramírez[2], Deyanira La Rosa Hernández[2,4], Martha Leonor Paradoa Pérez[2], Miriam SJB Lastre González[2], Laura María Reyes Díaz[2], Raúl Ramos Pupo[2], Zoila Estrella Medina Gondres[5]

[1]Escuela Latinoamericana de Medicina, La Habana, Cuba
[2]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[3]INOR, La Habana, Cuba
[4]Instituto de Gastroenterología, La Habana, Cuba
[5]Universidad de Ciencias Médicas de la Habana, Havana, Cuba
oliverperez@giron.sld.cu; irmav@giron.sld.cu; alina.alerm@infomed.sld.cu; carlosvillega@infomed.sld; victoria.gonzalez@infomed.sld.cu; deyani@infomed.sld.cu; mparadoa@infomed.sld.cu; oliver.perez@infomed.sld.cu; lreyez@giron.sld.cu; raulramos@infomed.sld.cu; zolila.medina@infomed.sld.cu

Keywords: Vaccines, traslational, Posgraduate, immunoresearch, immunoteaching

The Immunology is present in the formation of medical, biological (including biochemistry), pharmaceutical, and veterinary university students in Cuba. In Medicine, it is include in the second year as Immunology in the healthy human, a vaccinology course, and in immunopathology. A Cuban immunology book for the first part was already published1. The specialty of immunology is included in the medical postgraduate formation during 4 years with around 12 students by year from all the country and overseas. A new postgraduate program was already introduced in 20172. The formation is focused for their future immunologist´s work in four functions: medical attention; researchers; professors and administration which are not excluding areas. Cuba has a Society for Immunology that joint more than 1000 professionals working in areas related with Immunology. This is the biggest in Latinoamerica and it is represented in IUIS (International Union of Immunology Society) with 106 members. It belongs to the ALAI (Latinoamerican Association of Immunology) since 1996 and our Society organized ALAI meeting in 2002 and the next 2021 in Cuba. Taking in consideration our limitation in resources and to be speedier the Cuban researches are mainly focused in translational ones. In that sense, the immunologists are spread in all Cuban researcher centers mainly clustered in BioCubaPharma organization in Havana and their work in obtaining these goals are essential. Institutions work in collaboration for the production of human and veterinary prophylactic and therapeutic vaccines and products for human and veterinary health. As prevention is the main

gold on immunology, Cuba vaccinated against 18 infectious diseases, 13 in the national schedule of vaccination and 5 in risk population with coverage higher than 99%. This is guarantee by, the involvement of the community, the free of charge, and the national production of most vaccines. This strategy guarantee the close connection between immunology and biotechnology industry with public health and it has been successful in reducing infant mortality and infectious diseases in Cuba. The next challenge is to find treatments for chronic diseases of adulthood and to extend life expectancy3. More fundamental transition: the transformation of cancer from a rapidly fatal disease into a chronic condition4 is currently address particularly using immunotherapies against cancer, allergy, and neurodegenerative diseases.

REFERENCES

1. Pérez O e Veja I. Inmunología en el humano sano. Editora Ecimed 2017.
2. Pérez O et al. Programa de la Residencia en Inmunología, 2017.
3. Lage A. Connecting immunology research to public health: Cuban biotechnology. https://www.nature.com/articles/ni0208-109
4. Lage A and Crombet T. Control of Advanced Cancer: The Road to Chronicity. Int. J. Environ. Res. Public Health 2011, 8, 683-697; doi:10.3390/ijerph8030683

Neuroimmunological approach for evaluation of viral measles, mumps and rubella (MMR) triple vaccine

William Castillo-González, José Alejandro Rodríguez-Pérez, Eneida Barrios-Lamoth, Cristobal González-Losada, Alberto Juan Dorta Contreras

Universidad de Ciencias Médicas de la Habana, Havana, Cuba
williamcastillo@infomed.sld.cu; frcala@infomed.sld.cu; eneida@infomed.sld.cu; chriss.losada@gmail.com; adorta@infomed.sld.cu

Keywords: Antibodies, Measles, Mumps, Rubella, Vaccines, MMR vaccine, IgG intrathecal synthesis

Introduction: During the Neuroinflammation process there are a poly-specific and polyclonal activation in the cerebrospinal fluid. It means that there can be quantified antibodies against all the components of the vaccines may have received.

Materials and Methods: All the serum and cerebrospinal fluid (CSF) paired samples from pediatric patients with neurological symptoms that was submitted to a lumbar puncture were collected. Serum and CSF IgG, albumin were measured by immune-diffusion techniques using NOR and LC Partigen Immunoplates (Siemens, Marburg) and specific antibodies against measles, mumps and rubella were quantified by ELISA kits (Enzygnost, Siemens, Marburg). Reibergrams were employed in order to determine if there is IgG intrathecal synthesis. Later on, antibody index against the specific virus were calculated.

Results and Discussion: In all the neuroimmune inflammation process were found antibody index against measles, mumps and rubella in an ample confidence variation different among the different virus. Antibodies against mumps are significantly different from the other ones. It could be due to a natural different immune response or due to a deficient vaccine quality lot. Also it was possible to identify six pediatric patients that had no immune antibody index at all. It coincides with a immunodeficiency like infancy transient immunodeficiency in such patients.

Conclusions: This neuroimmunological approach can be used to evaluate the immune status in pediatric population.

Neutralizing antibodies induced by two recombinant BCG strain induces a protective immune response against human respiratory syncytial virus and human metapneumovirus

Alexis M Kalergis[1,2], Jorge A Soto[2], Nicolas M Galvez[2], Claudia A Rivera[2], Christian E Palavicino[2], Pablo F Céspedes[2], Emma Rey-Jurado[2], Susan M Bueno[2]

[1]Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[2]Facultad de Ciencias Biologicas, Pontificia Universidad Católica de Chile, Región Metropolitana, Chile
akalergis@bio.puc.cl; jasoto6@uc.cl; nrgalvez@uc.cl; carivercif@gmail.com; cpalavecino@gmail.com; pablocesdon@gmail.com; emmareyjurado@gmail.com; sbueno@bio.puc.cl

Keywords: Vaccine, respiratory disease, Humoral Immune Response, HRSV, Recombinant BCG, HMPV

Currently, both Human Respiratory Syncytial Virus (hRSV) and the Human Metapneumovirus (hMPV) are considered two major etiological agents for acute lower respiratory tract infections (ALRTIs) in children, elderly and immunocompromised people worldwide, promoting similar symptoms and clinical pathologies. Today, are not licensed vaccines for either of those viruses. Our study group developed two different recombinant Mycobacterium bovis Bacillus Calmette-Guérin (BCG) strains as vaccine candidates expressesing the hRSV Nucleoprotein (rBCG-N) or the hMPV Phosphoprotein (rBCG-P), respectively. Both vaccines are able to induce cellular protection against each respective virus. Here, we show that the humoral immune response induced by both rBCG-N and rBCG-P vaccines were able to protect against their viral infection promoting the secretion of specific antibodies against each virus, and several specific proteins through classical linked recognition. Also, we identified that both vaccines promoted the induction of neutralizing antibody in vitro, as well as sera transfer protection in vivo. Our results support the notion that the use of a recombinant BCG vaccines could be considered as a new platform against these two major respiratory pathogens.

Polysaccharides from bacteria and commensals as possible mucosal vaccine and adjuvants

Laura María Reyes Díaz[1], Miriam SJB Lastre González[1], Carlos Bauman[2], Yusnaby Borrego González[1], Dainerys A Pérez Lastre[3], Raúl Ramos Pupo[1], Victoria E González Ramírez[1], Osmir Cabrera Blanco[4], Alexander Batista Duharte[5], Oliver Pérez[1,6]

[1]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[2]Facultad de Ciencias Médicas, Universidad Nacional de Rosario, Rosario, Argentina
[3]Hospital Vitória, Unidade Avançada São José Dos Pinhais, Curitiba, Brazil
[4]Instituto Finlay (IF), La Habana, Cuba
[5]School of Pharmaceutical Sciences, Araraquara, Brazil
[6]Escuela Latinoamericana de Medicina, La Habana, Cuba
lreyes@giron.sld.cu; oliver.perez@infome.sld.cu; carlosbauman@gmail.com; yusnaby@giron.sld.cu; dperezlastre@gmail.com; raulramos@infomed.sld.cu; victoria.gonzalez@infomed.sld.cu; ocabrera@finlay.sld.cu; batistaduhartea@gmail.com; oliverperez@giron.sld.cu

Keywords: Bacillus subtilis, Neisseria, Polysaccharides, cross-reactivity, commensals, vaccine adjuvants, Mucosal IgA

Introduction: Polysaccharides are thymus-independent type 2 antigens (TI-2)1 inducing mainly IgM with limited or absence of class switching, affinity maturation, and immunological memory2,3. They are virulent factors of encapsulated bacteria3. Consequently, polysaccharides did not work in children under 2 years old4and the conjugate vaccines overcome these problems5.Commensal are decisive in the induction of mucosal immune response. These enteric bacteria engender a self-limiting IgA mucosal immune response while permanently colonizing the gut6, 7.Most infection arrived or establish at mucosal surface where secretory (S)IgA is the main protector antibody. SIgA is inducing as TD (dependent) or TI immune responses mainly against proteins and polysaccharides, respectively. Protection could be inducing by crossreactive immune responses against the same or similar proteinscoming from pathogenic and non-pathogenic Neisseria. Nevertheless, polysaccharide´scrossreactivity are less considered. N. meningitidis has 13 serogroups base in polysaccharide composition of their capsule. The A, B, C, Y, and W135 serogroups were the most pathogenic ones. In addition, polysaccharidesstimulate mainly the innate immune response like Tgd and B1 lymphocytes8. Consequently, some SIgA probably stimulated by commensal and carriage could be crossprotective. Bacillus subtilisis a gram-positive probiotic bacterium. Nattō is a traditional Japanese food made from soybeans fermented with B. subtilis var. natto9. This produces an exopolysaccharide10. AFCo (Adjuvant Finlay Cochleate) 1 is derived from AFPL®1 which works by mucosal route11,12,13.

Lastly, most vaccines are applied by parenteral route. The mucosal vaccination routeis superior toparenteral one because it induce systemic immune response like parenteral did but in addition, it stimulate mucosal immune response14.However, the use of polysaccharides as vaccine antigensby mucosal route was less explored. Consequently we hypothesizedthat polysaccharides may induce mucosal protective immune response by crossreactivity.

Aims: To determine if there is crossed mucosae reactivity between proteins of different species and pathogen serogroups and commensals of the generous Neisseria;to determine the existence of IgA crossreactivity between polysaccharides of different serogroups of pathogenic Neisseria; to determine the crossreactivity induced by adjuvantated formulation over polysaccharide, and to explore the adjuvant effect of B. subtilis over polysaccharide antigens.

Methods: Western Blot and ELISA to determine the specific salival IgA of polysaccharide vaccinated human or mice were used. AFCo1 or B. subtilis spores plus co-administered polysaccharides for intranasal route were used. ELISPOT to determine IL-12 secretory cells was used.

Results and Discussion: Several structural homology was found between proteins of pathogen and commensal species of Neisseria. Cross-reactive response anti-proteins of pathogens and commensal Neisseria were determined at IgG and IgA level (Fig. 1 and 2). Cross-reactive anti-polysaccharide A, C, Y, and W135 responses were obtained only at IgA level (Fig. 3 and 4) but not at the IgG ones (data not shown). IL-12 secretory cells were induced using B. subtilis spores (death or alive) as well as with AFCo1 adjuvant (Fig. 5). Many of the proteins present in the pathogenic species of the genus Neisseria are also found in commensal species that inhabit our body, one of the most significant is PorB (class2/3), meningococcal protein), which depending of the strain can be formed by PorB2 or PorB315, proteins that present antigenic crossreactivity, inducing a high production of opsonophagocytic and/or bactericidal antibodies16,17. The non-pathogenic species evaluated in this work are commensal that are part of the normal microbiotra of the human nasopharynx as is the case of N. lactamica and N. flava18. So, the immune system is in direct and continuous contact with both, and with greater emphasis the mucosal system must have a wide response against these pathogens to be constantly stimulated with these antigens, particularly in these sites IgA is the dominant immunoglobulin and the most abundant, it is said to be 10 times the concentration of IgG19. It has an important role in the protection against infections in the upper respiratory tract and genital, against bacterial pathogens such as N.

Neisseria					
meningitidis			gonorrhoeae	lactamica	Flava
Serogroups					
B	A	C			
HmbR	96	-	94	-	-
FrpB	-	93	96	97	-
TbpA	94	76	93	97	?
TbpB	71	40	54	75	?
PorA	93	65	-	-	?
PorB	93	90	65	71	46
rmpM	98	98	94	89	75
fHbp	98	98	94	89	75
Opc	99	98	44	-	?
Opa	-	-	68	71	?

FIGURE 1: Protein crossr eactivity between serogroups of *Neisseria meningitidis*, *N. gonorrhoeae* and some commensal *Neisseria* species. B, A, and C are N. meningitidis serogroups A, B and C, respectively; Ng *N. gonorrhoeae*; NL, *N. lactamica*; Nf, *Nflava*; ?, notfound.

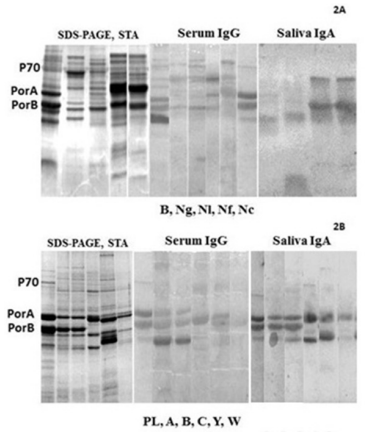


FIGURE 2: Protein crossreactirity between serogroups and species of *Neisseria*. A Protein crossreactivity between species of *Neisseria*; B, *N. meningitdis serogroup*; Ng, *N. gonorrhoeae*, and some commensal *Neisseria* species (Nl, *N. lactamica*; NF, *N. flava* and Nc *N. catarralis*, B, Protein crossreactivity between serogroups of *N. meningitidis* serogroups A, B, C, Y and W).

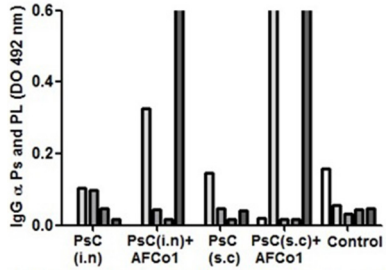


FIGURE 3: Cross reactive anti polysaccharid e A, C, Y, and W_{135} were not obtained at IgG level where each group only induces specific IgG. Ps, polysaccharide; AFCo1 is adjuvant with PL (outer membrane vesicle) of the Cuban meningococcal vaccine; i.n, intranasal; s.c, subcutaneous; +, co-administered.

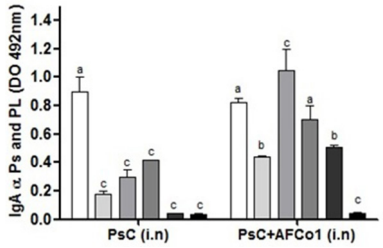


FIGURE 4: Cross reactive anti polysaccharides of *Neisseria meningitidis* serogroup A , C, Y, and W_{135} IgA responses. Ps, polysaccharide; AFCo1 is adjuvant with PL; i.n, intranasal; +, co-administered.

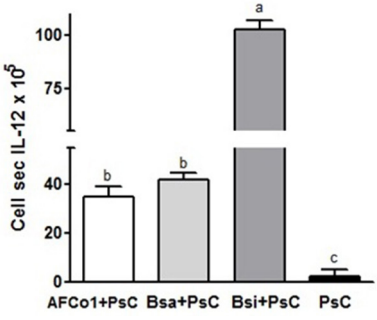


FIGURE 5: Determination of the number of IL-12 secretory cells induced by immunization with AFCo1 or *Bacillus subtilis* spores combined with PsC by the i.n route. PsC, polysaccharide of *N. meningitidis* serogroup C; AFCo1 is adjuvant with PL (outer membrane vesicle) of the Cuban meningococcal vaccine; Bsa, *B. subtilis* spores alive; Bsi, *B. subtilis* spores inactivated.

meningitidis and N. gonhorroeae20. It has been postulated that the SIgA induces more crossed reactivity than serum IgG21. IL-12 is produced by monocytes, macrophages, dendritic cells, neutrophils and B cells. Its main functions are to induce the activation of T and NK cells, favoring the production of IFN-γ and increasing cytotoxic activity. In addition, it induces the immunpolarization of CD4+ T cells towards Th1 subset22. The induction of high concentration of IL-12 by the adjuvanted formulations favors the regulations of the adaptive response, since it plays a central role in coordinating innate and acquired immunity. Various MAMP (microbial-associated molecular pattern) derived from microorganisms have been known to regulate immune responses23. In other works it found that the presence of component of B. subtilis plays a role in regulation the immunepolarization of Th1 versus Th2 subset and has adjuvant effect like poly γ glutamic acid24. Our preliminary results suggest the potential of inactivated or alive spores of B. subtilis reacting as an adjuvant over polysaccharide of N. meningitidis serogroups C. On the other hand it seems that the involved mechanism in this adjuvant capacity of alive and inactivated spores is not similar.

Conclusions: It seems that there is mucosal crossreactivity over proteins and in addition to bacterial polysaccharides and commensal of species of Neisseria and the adjuvant effect of spores of B. subtilis over polysaccharide was determined.

REFERENCES

1. Weintraub A. Immunology of bacterial polysaccharide antigens. Carbohydr Res (2003) 338:2539-47. doi:10.1016/j.carres.2003.07.008.
2. Jakobsen H, Jonsdottir I. Mucosal vaccination against encapsulated respiratory bacteria - new potentials for conjugate vaccines? Scand J Immunol 2003; 58:119-28. doi:10.1046/j.1365-3083.2003.01292.
3. Mbow ML, E De Gregorio, NM Valiante, R Rappuoli. New adjuvants for human vaccines. CurrOpinImmunol, 2010; 22: 411-416.
4. Pollard AJ, KP Perrett, PCBeverleyMaintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. Nat Rev Immunol, 2009; 9: 213-220.
5. Pollard AJ, KP Perrett, PC Beverley. Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. Nat RevImmunol, 2009; 9: 213-220.
6. Shroff KE, Meslin K, Cebra JJ. Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut.Infect Immun. 1995; 63(10):3904-13.
7. P Brandtzaeg, H Kiyono, R Pabst &M W Russell. 2008. Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunology volume 1, pages 31–37 (2008) doi:10.1038/mi.2007.9
8. Pérez O y Vega I. Inmunología en el sujeto sano. EciMed 2017.
9. Hosking R (1995). A Dictionary of Japanese Food - Ingredients and Culture. Tuttle. p. 106. ISBN 0-8048-2042-2.
10. ElsholzAKW, WackerSA, and LosickR. Self-regulation of exopolysaccharide production in Bacillus subtilis by a tyrosine kinase. Genes & Development 28:1710-1720. doi/10.1101/gad.246397.114.
11. Pérez O, Bracho G, Lastre M, del Campo J, Gil D, Zayas C, Acevedo R, González D, López JA, Taboada C, Solis RL. Novel adjuvant based on a proteoliposome-derived cochleate structure containing native lipopolysaccharide as a pathogen-associated molecular pattern. Immunology and Cell Biology 2004; 82,603–610.
12. Pérez O, Lastre M, Cabrera O, del Campo J, Bracho G, Cuello M, Balboa J, Acevedo R, Zayas C, Gil D, Mora N, González D, Pérez R, González E, Barberá R, Fajardo EM, Sierra G, Solís RL, and Campa C. New vaccinesrequirepotentadjuvantslike AFPL1 and AFCo1. Scand J Immunology 2007; 66:271-77.
13. Pérez O, Batista-Duharte A, González E, Zayas C, Balboa J, CuelloM, Cabrera O, Lastre M and Schijns VEJC. Human prophylactic vaccine adjuvants and their determinant role in new vaccine formulations. Brazilian J Med and Biol Res (2012) 45(8):681-692.
14. Mi-NaKweon. Sublingual mucosa: A new vaccination route for systemic and mucosal immunity. Cytokine, 2011; 54: 1-5.
15. Minetti, C.A.S.A., Blake, M.S., Remeta, D. (1998) Characterization of the structure, function and conformational stability of PorB class 3 protein of Neisseria meningitidis. J. Biol. Chem. 273, 25329–25338
16. Guttormsen, H.K., Wetzler, L.M., Næss, A. (1993) Humoral immune response to class 3 outer membrane protein during the course of meningococcal disease. Infect. Immun. 61, 4734–4742.
17. Claassen, I., Meylis, J., van der Ley, P., Peeters, C., Brons, H., Robert, J., Borsboom, D., van der Ark, A., van Straaten, I., Roholl, P., Kuipers, B., Poolman, J. (1996) Production, characterization and control of a Neisseria meningitidis hexavalent class 1 outer embrane protein containing vesicle vaccine. Vaccine 14, 1001–1008.
18. Coen PG, Cartwright K, Stuart J. Mathematical modelling of infection and disease due to Neisseria meningitidis and Neisseria lactamica. Int J Epidemiol 2000; 29: 180–188.
19. Kerr M. The strucrue and function of human IgA. Biochem J 1991; 271: 285–296.
20. Zhang Q, Choo S, Everard J. Mucosal immune responses to meningococcal group C conjugate and group A and C polysaccharide vaccines in adolescents. Infect Immun 2000; 68: 2692–2697.

21. AJ Macpherson, K D McCoy, F-E Johansen & P Brandtzaeg.2008. The immune geography of IgA induction and function. Mucosal Immunology, volume 1, pages 11–22.
22. Watford WT, M Moriguchi, A Morinobu, JJ O'Shea. 2003 The biology of IL-12: coordinating innate and adaptive immune responses Cyt Growth Fact Rev, 14: 361–368.
23. Akira, S. and Takeda, K. 2004. Toll like receptor signaling. Nat. Rev. Immunol. 4:499
24. Sunghoon K, J Youn Yang, Kyuheon L, K Heon, M Gi, J Mogg, D Jin, S Hong and J Youn. (2009). Bacillus subtilis-specific poly- γ-glutamic acid regulates development pathways of naive CD4+ T cells through antigen-presenting cell-dependent and –independent mechanisms. Int. Immun, 21:977-990.

Anti-idiotypic monoclonal antibody 10.D7, mimicking vascular endothelium growth factor (VEGF), inhibits metastasis formation

Tábata De Almeida Da Silva, Gabriel Esquitini Machado, Bárbara Hamaguchi, Rodrigo Barbosa Aguiar, JANE ZVEITER DE MORAES

Federal University of São Paulo, São Paulo, Brazil
tabata25almeida@gmail.com; gabriel.esquitini@gmail.com; barbara.hamaguchi@gmail.com; rodrigo.aguiar@uol.com.br; janezvmo@gmail.com

Keywords: Vaccination, Vascular Endothelial Growth Factor A, metastasis, monoclonal antibodies, Idiotype

Melanoma is the most aggressive skin tumor. Conventional treatments, besides having side effects, are not efficient, which makes imperative the seek for new treatment options. Immunotherapy has been considered as an alternative, and antiangiogenic approaches are among the most promising ones. Vascular endothelial growth factor (VEGF) plays an essential role in the angiogenic process. Bevacizumab is a humanized anti-VEGF monoclonal antibody that has been already approved for the treatment of a variety of tumors. However, undesirable side effects that impair its clinical use have been reported. We have described an anti-bevacizumab idiotype (Id) mAb, 10.D7, that presented encouraging results when used as immunogen. Previous proof-of-concept experiments showed significant delay in tumor growth in animals that received tumor implantation after being immunized with anti-Id mAb 10.D7. In the present study, we analyze the potential of mAb 10.D7 vaccination to inhibit metastatic tumor formation. For that, purified antibody, conjugated to keyhole limpet hemocyanin (KLH) and later incorporated into incomplete Freund's adjuvant (AIF), was used twice as immunogen in C57Bl/6 mice, before systemic B16F10 tumor induction by the caudal route. Titration of serum VEGF-binding antibodies was performed at different times of the experiment. On the 21st day after the challenge with melanoma cells, the animals were euthanized. Surgical removal of the lungs and macroscopic counting of the metastatic foci on pulmonary surface were performed. After this, lungs were fixed in formaldehyde 3.7% for 24 hours and, then, dehydrated with ethanol 70%, and paraffin embedded. Lung sections were stained with hematoxylin and eosin (HE); images were acquired using a light microscope and analyzed by ImageJ software. The results showed increased serum levels of VEGF-binding antibodies throughout the experiment, with the following O.D. ± SEM values obtained in each group on the last day of the experiment: mAb 10.D7, 0.2580 ± 0.017; isotype control, 0.0750 ± 0.017; and adjuvant control, O.D. 0.0708 ± SEM 0.017 (two-way ANOVA/Tuckey post-test, $p<0.0001$ compared to controls). At the end of the metastasis induction assay, lungs from animals immunized with 10.D7

mAb showed fewer metastatic foci (4.41 ± SEM 5.272), compared to the obtained with control groups. Mouse immunization with isotype control mAb and adjuvant alone led to 19.78 ± SEM 5.940 and 32.50 ± SEM 6.117 metastatic foci, respectively. N=8/group. Histological analyses corroborated the macroscopic findings, since the percentage of area occupied by metastatic foci in the 10.D7-immunized group (10.80 ± SEM 3.912) was statistically lower than the obtained with control groups (isotype control mAb group, 30.25 ± SE 4.149; adjuvant group, 36.60 ± SEM 4.149) (one-way ANOVA, $p<0.001$). The findings suggest that immunization with mAb 10.D7 interferes in the metastasis formation. This indicates the potential of mAb 10.D7 to contribute with the development of more efficient protocols to prevent tumor recurrence, since the immunization induces T cell-dependent response and consequent long-term memory.

Evaluation of the immunogenicity of a new pneumococcal conjugate vaccine (PCV10) administered concomitantly with the Heberpenta®-L vaccine in rabbits

Yosbany Pérez Barrios, Abel Zanuy, Mabel Hernandez Cedeño, Rocmira Perez, Yisabel Aranguren, Caridad Zayas, Reinaldo Acevedo, Alex Quintero, Darcy Nunez, Yury Valdes Balbin, Darielys Santana-Medero, Dagmar Garcia Rivera, Vicente Verez Bencomo

Instituto Finlay (IF), La Habana, Cuba

ypbarrios@finlay.edu.cu; azanuy@finlay.edu.cu; mahernandez@finlay.edu.cu; rpnicado@finlay.edu.cu; yaranguren@finlay.edu.cu; czayas@finlay.edu.cu; racevedo@finlay.edu.cu; aquintero@finlay.edu.cu; dnunez@finlay.edu.cu; yvbalbin@finlay.edu.cu; dsantana@finlay.edu.cu; dagarcia@finlay.edu.cu; vicente.verez@finlay.edu.cu

Keywords: Vaccine, interference, immune response, Pneumococcal vaccine, Concomitance

Finlay Institute of Vaccines is developing a heptavalent conjugate vaccine (PCV7-TT) for childhood against serotypes 1, 5, 6B, 14, 18C, 19F and 23F of Streptococcus pneumoniae. This vaccine is next to be approved by the National Regulatory Authority of Cuba (CECMED) to apply first in children from 1 to 5 years of age and later for infants. As second generation of vaccine, a new decavalent vaccine candidate (PCV10-TT) is under research looking for wider coverage of serotypes in infants and better market opportunities. The new vaccine candidate will replace the PCV7-TTadding three new serotypes: 6A, 19A and 9V conjugated to tetanus toxoid. Good immunization practices require concomitance studies of new candidates with current applied vaccines. The interactions between concomitant vaccines may cause an increase, or in the worst case, a decrease in the immunogenicity of one or more components of the vaccines applied. The aim of this work was to evaluate the possible immunological interference between the concomitant administrations of PCV10-TT candidate with the Heberpenta®-L, a DTP combined vaccine, in the New Zealand rabbit model. Prevnar 13® was used as a control vaccine to compare vs. PCV10-TT candidate. Rabbits were immunized concomitantly with three doses of Heberpenta®-L and Prevnar 13® or PCV10 -TT. The IgG antibody responses to all antigens were evaluated by immunoenzymatic assays. No interference were induced by PCV10-TT on the humoral response against the antigens present in the Heberpenta®-L vaccine ($p > 0.05$). The co-administration did not induce a reduction of the immune response generated against the pneumococcal polysaccharides contained in the PCV10-TT vaccine ($p > 0.05$). The concomitant administration

of Heberpenta®-L did not induce interference on Prevnar 13® antigens. The PCV10-TT response behaved similar to the Prevnar 13® vaccine when they were applied concomitantly with Heberpenta®-L. The preclinical results suggest that PCV10-TT will not interfere with the immune response induced by the licensed Heberpenta-L® vaccine.

Alternatives to multiple doses of allergy´s immunotherapy

Miriam SJB Lastre González[1], Yaxunaris Pérez Fumero[1], Laura María Reyes Díaz[1], Wendy Ramirez González[2], Mary Carmen Reyes Zamora[2], Yusnaby Borrego González[1], Dainerys A Pérez Lastre[3], Raúl Ramos Pupo[1], Alexis Labrada Rosado[2], Roberto R Grau[4], Alexander Batista Duharte[5], Oliver Pérez[1,6]

[1]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba

[2]Centro Nacional de Biopreparados, Havana, Cuba

[3]São José Dos Pinhais, Rua Sete de Setembro, 290-Centro PR, Curitiba, Brazil

[4]National University of Rosario, Rosario, Argentina

[5]Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista Júlio de Mesquita Filho, Araraquara, Brazil

[6]Escuela Latinoamericana de Medicina, La Habana, Cuba

oliver.perez@infomed.sld.cu; ypfumero@infomed.sld.cu; lreyes@giron.sld.cu; wendy@biocen.cu; mcarmen.reyes@biocen.cu; yusnaby@giron.sld.cu; dperezlastre@gmail.com; raulramos@infomed.sld.cu; labrada@biocen.cu; robertograu.conicet@gmail.com; batistaduhartea@gmail.com; oliverperez@giron.sld.cu

Keywords: Immunotherapy, Vaccines, allergy, SinTimVaS, Single Time Vaccination Strategy

Introduction: Immunogenicity is the capacity of an antigen/allergen to induce the immune response by it one during the first contact. It depends of host and antigen factors. The host dependence involves: 1, the genetic constitution where the immune response depends of the polymorphis and polygenes of the MPP (peptide presenting molecules)-II and MPP-I and the population could be classified in: responder, hyperresponder and no responder; and 2, the life's periods and sex: gestation, neonate and infancy, and senescent. The antigen dependence involves the: hexogen character (xeno- > alo- > auto-antigens); chemical structure (protein > saccharide); molecular structure (particulate > soluble); complexity (globular proteins > degraded); optimal concentration; optimal doses; interval between doses; adjuvant and route (parenteral or mucosa)1,2. We developed AFPL®1 (Adjuvant Finlay proteoliposome 1) derived from Neisseria meningitides serogroups B and an AFCo1 (Adjuvant Finlay Cochleate 1) derived from PL 1. Both contain multiple MAMP (microbial associated molecular patters), mainly LPS (TLR4 agonist) and porins (TLR2 agonist) which induces a Th1 (IL12/IFNg, delayed type-hypersensitivity and not IL4 neither specific IgE) immunepolarization3,4,5,6,7. Vallergen-Ds is an efficient immunotherapy against Dermatophagoides siboney allergen sensitization used by subcutaneous or sublingual routes developed by BioCen, Mayabeque, Cuba. In addition, the SinTimVaS (STVS,

single time vaccination strategy) which combine simultaneously two primes (one parenteral and another mucosal) was developed8,9,10. We hypothesized that adjuvant and route could be determinant for allergy´s immunotherapy.

Aims: to explore the reduction of doses using AFPL®1 in combination with D. siboney allergen adsorbed onto aluminum hydroxide (Alum, named Prolinem-Ds) and to explore SinTimVaS using parenteral Prolinem-Ds and mucosal Vallergen-Ds.

Results: The anti-allergic pharmaceutical composition and methods for the preparing thereof (Prolinem-Ds) was proprietary11,12,13. AFPL®1 was extensively used in human (more than 65 x 106 human doses and 12 x 106 children doses) as part of national Cuban vaccination schedule and overseas. The allergen immunotherapy required more than 40 doses sparse in several years. Using AFPL®1 plus D. siboney allergen extract adsorbed onto alum and only three doses by parenteral (intramuscular or subcutaneously) the Th2 allergen patter was overcome to a Th1 one and a reduction in total and anti-allergen IgE was observed12. The pre-clinical toxicological evaluation of Prolinem-Ds in preventative and therapeutic model was satisfactory13. The phase I (safety, tolerability and immunogenicity) in 20 sensitized to D. siboney patients (http://registroclinico.sld.cu/, RPCEC00000139) was already conducted. Prolinem-Ds in three subcutaneously doses was well tolerated and induces changes at antibody, cellular, and dermal responses where IgE/IgG4, IL-10, and IFNg seen to be relevant markers. The development of SinTimVaS to: reduce time-cost of vaccination, increase coverage, induce systemic and mucosal immune response, and target herd immunity by the combination of a new priming boost strategy that use two doses (one parenteral and another mucosal) simultaneously was conducted8,9,10. The function and adjuvant immunopolarization

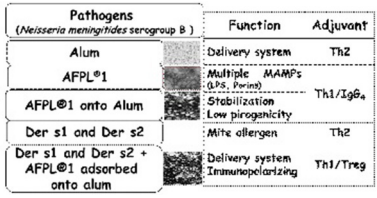


FIGURE 1: Composition, function and adjuvant activity of anti allergen vaccine (Prolinen-Ds). Alum, aluminum hydroxide; AFPL®1; adjuvant Finlay proteoliposome 1; Der s, *Dermatophagoides siboney* allergen; MAMP, microbial associated molecular patters; LPS, lipopolysaccharide; Th, T helper cells;Treg, T regulatory cells.

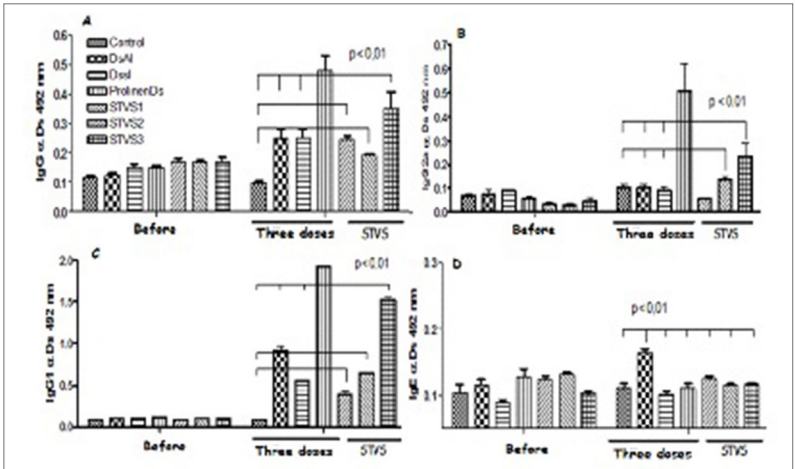


FIGURE 2: Immune response induce by Prolinem-Ds. DsA1, *Dermatophagoides siboney* allergen in Alum; Dssl, *D. siboney* allergen sublingual; STVS, SinTimVas.

of the formulation is summarized in Fig. 1 where a Th1 and Treg response was obtained. Vallergen-Ds sublingual plus Prolinem-Ds subcutaneously simultaneously in 1 (STVS1), 2 (STVS2), or 3 (STVS3) times were used. The induction of specific IgG was achieved by 2 or 3 doses of either the controls or STVS. The betters specific IgG responses were obtained with Prolinem-Ds and STVS3; but good IgG response were also observed with STVS1 and STVS2 (Fig. 2A). The IgG2a as Th1 driving response was only induce by Prolinem-Ds and STVS3 (Fig. 2B). The specific IgE response was only induced by DsAl as positive control (Fig. 2D). The allergen challenge was also conducted and the evaluations at 24 h of 8 days later maintain the Th1 and Treg polarization (data not shown).

Conclusions: Prolinem-Ds functions reducing the doses from more than 40 to only 3 ones, at least in preclinical and phase I clinical trial. SinTimVaS may reduce the doses still more. More work is needed to determine the mucosal response.

REFERENCES

1. Pérez O y Vega I. Inmunología en el sujeto sano. EciMed 2017.
2. O Pérez, A Batista-Duharte, E González C Zayas, J Balboa, M Cuello, O Cabrera, M Lastre and VEJC Schijns. Human prophylactic vaccine adjuvants and their determinant role in new vaccine formulations. Brazilian J Med and Biol Res (2012) 45(8):681-692.

3. Pérez O, Bracho G, Lastre M, del Campo J, Gil D, Zayas C, Acevedo R, González D, López JA, Taboada C, Solis RL. Novel adjuvant based on a proteoliposome-derived cochleate structure containing native lipopolysaccharide as a pathogen-associated molecular pattern. Imm Cell Biol 2004, 82,603–610.
4. Pérez O, Lastre M, Bracho G, del Campo J, Zayas C, Acevedo R, Gil D, Mora N, González D, Balboa J, Cabrera O, Cuello C, Pérez DR, Barberá R, Fajardo EF, Sierra G, Solís RL and Campa C. Natural Neissera derive proteoliposome and cochleate as potent vaccine adjuvants. PharmacologyOnline 3:762-64, 2006.
5. Pérez O, Lastre M, Cabrera O, del Campo J, Bracho G, Cuello M, Balboa J, Acevedo R, Zayas C, Gil D, Mora N, González D, Pérez R, González E, Barberá R, Fajardo EM, Sierra G, Solís RL and Campa C. New vaccines require potent adjuvants like AFPL1 and AFCo1. Scand J Immunology 2007, 66:271-77.
6. Pérez O, Lastre M. 2013. Proteoliposome: VA-MENGOC-BC® core and adjuvant platform. VacciMonitor. 2013. 22:1–3.
7. Pérez O, Romeu B, Cabrera O, González E, Batista-Duharte A, Labrada A, Pérez R, Reyes L, Ramírez W, Sifontes S, et al. Adjuvants are key factors for the development of future vaccines: Lessons from the Finlay adjuvant platform. Front Immunol. 2013; 4:1-12.
8. Pérez O, González E, Romeu B, del Campo J, Acevedo R, Lastre M, et al. Vacunas unitemporales. OCPI, CU/P/2008/215.
9. Elizabeth González y cols. Single Time Vaccination Strategy. VacciMonitor 2009; 18(2):75-77 90.
10. Pérez Martín Oliver G, González Aznar Elizabeth, Romeu Alvarez Belkis, del Campo Alonso Judith M, Acevedo Grogues Reinaldo, Lastre González Miriam de SJB, Zayas Vignier Caridad, Cuello Pérez Maribel, Cabrera Blanco Osmir , Nuñez Gutierrez Niury, Balboa González Julio A. Single-Time Vaccines. WO 2010/057447 (27.05.2010 Gazatte 2010/21).
11. Lastre M, Pérez O, Labrada A et al. Método de obtención de estructuras cocleares. Composiciones vacunales y adyuvantes basados en estructuras cocleares y sus intermediarios. Conceded: OCPI 2008
12. Lastre M, Pérez O, Labrada A, Bidot I, Perez J, Bracho G, et al. Bacterial derived proteoliposome for allergy vaccines. Vaccine 2006; 24 (Suppl 2): S2-S5.
13. Lastre M, Pérez O, Labrada A et al. PCT/CU 03/00007. Antiallergic pharmaceutical composition and methods for the preparing thereof. US Patent 7648709 (2010); European Patent EP 1 520 587 B1 (2011).
14. Wendy Ramírez, Virgilio Bourg, Damaris Torralba, Elisa Facenda, Beatriz. Tamargo, Bárbara O. González, Gustavo Sierra, Oliver Pérez, Yordanis Perez-Llano & Alexis Labrada. Safety of a proteoliposome from Neisseria meningitides as adjuvant for a house dust mite allergy vaccine. J Immunotoxicology, 2017;14(10):152–159. doi.org/10.1080/1547691X.2017.1346007.

Adaptive immune gene expression induced by an inactivated infectious pancreatic necrosis virus in rainbow trout (Oncorhynchus mykiss)

Catalina Tufiño-Loza[1], Carolina Tafalla Piñeiro[2], Constantino López-Macías[3,4], Edith Rojas-Anaya[5], Celene Salgado-Miranda[6], Elizabeth Loza-Rubio[5]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Centro de Investigación en Sanidad Animal (CISA), INIA, Valdeolmos, Spain
[3]Nuffield Department of Medicine, Medical Sciences Division, University of Oxford, Headington, United Kingdom
[4]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[5]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Jiutepec, Mexico
[6]Universidad Autónoma del Estado de México, Toluca, Mexico
hidra1983@gmail.com; tafalla@inia.es; constantino@sminmunologia.mx; edith_ra23@hotmail.com; celene_salgado@yahoo.com; loza.elizabeth@inifap.gob.mx

Keywords: Vaccines, gamma irradiation2, adaptive immune response3, IPNV4, trout5

Infectious pancreatic necrosis virus (IPNV) constitutes one of the major concerns for aquaculture, thus, the importance of making effective and safe vaccines against this virus. In the current work, we have evaluated the immune expression of relevant genes induced in rainbow trout (Oncorhynchus mykiss) by an experimental vaccine against IPNV administered in the presence or absence of Salmonella Typhi porins as adjuvant. For this, we tested three formulations: inactivated IPNV and porins either alone or combined with the inactivated virus. The immune response was assessed in rainbow trout by transcriptional kinetics of several genes after vaccination and through the protection that is conferred by the challenge. This study revealed that the inactivated IPNV induced predominantly a humoral immune response, while the porins either alone or combined promoted mainly a cell mediated immune response and showed a lower protection. The inactivated viral vaccine demonstrated its potential inducing a moderate protective effect against IPNV in rainbow trout, whereas the porins showed their capacity to enhance the immune response as long as an adequate dose is provided.

Conservation and immunogenicity of the porin OmpC among typhoidal and non-typhoidal Salmonella serovars

Nuriban Valero-Pacheco[1,2], Joshua Blight[3], Marisol Pérez-Toledo[1,2], Gustavo Aldapa-Vega[1,2], Isabel Wong-Baeza[1], Ayako Kurioka[4], Christian Pérez-Shibayama[5], Cristina Gil Cruz[5], Luvia Enid Sánchez-Torres[1], Rodolfo Pastelin-palacios[6], Armando Isibasi[2], Arturo Reyes-Sandoval[3], Paul Klenerman[4], Constantino López-Macías[2,3]

[1]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Nuffield Department of Medicine, Medical Sciences Division, University of Oxford, Headington, United Kingdom
[4]Peter Medawar Building for Pathogen Research, University of Oxford, Oxford, United Kingdom
[5]Kantonsspital St. Gallen, St. Gallen, Switzerland
[6]Departamento de Biología, Facultad de Quimica, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
nuribanv@gmail.com; j.blight@imperial.ac.uk; marisolpereztoledo@gmail.com; g.aldapa.v@gmail.com; isa_wb@yahoo.com.mx; ayako.kurioka@ndm.ox.ac.uk; christian.perez@kssg.ch; Cristina.gil@kssg.ch; luviasanchez@hotmail.com; rodolfop@unam.mx; isibasi@prodigy.net.mx; arturo.reyes@ndm.ox.ac.uk; paul.klenerman@medawar.ox.ac.uk; constantino@sminmunologia.mx

Keywords: Porins, conservation, Immunogenicity, OmpC, typhoidal and non-typhoidal salmonella

Salmonella enterica infections remain a challenging health issue, causing significant morbidity and mortality worldwide. The current vaccines against typhoid fever display moderate efficacy, while no licensed vaccines against paratyphoid fever or invasive non-typhoidal salmonellosis are available. Therefore, there is an urgent need to develop multivalent vaccines that can protect against typhoidal and non-typhoidal Salmonella infections. Our previous studies have shown that Salmonella porins OmpC and OmpF are highly immunogenic and protective antigens that efficiently elicit protective antibody and cellular immunity against Salmonella. The porin OmpC, has a high degree of homology in terms of sequence and structure among Enterobacteriaceae porins, however, it remains unknown the degree of conservation of this porin among typhoidal and non-typhoidal Salmonella serovars, and whether these conserved sequences could also be immunogenic. Through bioinformatics, we found that the porin OmpC contains six distinct amino acid sequences, which are highly conserved among typhoidal and non-typhoidal Salmonella serovars. These conserved sequences are located mostly along the transmembrane domains of the porin β-barrel. Furthermore, we found that some of the conserved OmpC sequences are also immunogenic to human CD4+ T cells.

Considering that porins are highly immunogenic and protective vaccine candidates against Salmonella, our findings may lead to better understanding the basis of antigen specificity of Salmonella porins, which could have direct implications for the rational design of multivalent vaccines against Salmonella.

Immunization with Salmonella porins induces innate lymphoid cell expansion and selective features of the T cell response to live Salmonella in draining lymph nodes

Marisol Pérez-Toledo[1], David R. Withers[1], Adriana Flores-Langarica[1], Nuriban Valero-Pacheco[2], Nick D. Jones[1], Rodolfo Pastelin-palacios[3], Constantino López-Macías[4,5], Adam F. Cunningham[1]

[1]Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom
[2]Center for Immunity and Inflammation, New Jersey Medical School - Rutgers Biomedical and Health Sciences, The State University of New Jersey, New Jersey, United States
[3]Departamento de Biología, Facultad de Quimica, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[4]University of Oxford, Oxford, United Kingdom
[5]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
marisolpereztoledo@gmail.com; d.withers@bham.ac.uk; a.floreslangarica@bham.ac.uk; nuribanv@gmail.com; n.d.jones@bham.ac.uk; rodolfop@unam.mx; constantino@sminmunologia.mx; a.f.cunningham@bham.ac.uk

Keywords: Porins, Salmonella, draining lymph nodes, T cell response, innate lymphoid cells expansion

After infection with Gram-negative bacteria like Salmonella, Th1 and Th17 responses, but not Th2 responses, are detectable, as measured through the induction of cytokines such as IFN-gamma, IL-17 and IL-4 respectively. What is unclear is the relative contribution of individual antigens to this cytokine response. In this study we have immunized mice with live Salmonella enterica, or purified, soluble flagellin (FliC) or purified porin proteins (OmpF and OmpC) (porins are used as vaccine candidate for typhoid fever and for non-typhoidal Salmonellosis) and compared the immune responses induced. As expected, Salmonella induces IFN-gamma and IL-17, and FliC induces IL-4 and IL-17, but not IFN-gamma. Surprisingly, porins induced the production of all of these cytokines, along with IL-22. After immunization with porins and live Salmonella, there was a pronounced leucocytosis in the draining lymph node, accompanied by increased numbers of innate lymphoid cells type 1-3. Additionally, porins induced IFN-gamma in both CD4 and CD8 T cells, at levels comparable to that observed after live bacteria. The production of IFN-gamma from CD4 T cells, but not CD8 T cells, after porin immunization was dependent upon T-bet. Therefore, purified bacterial proteins can induce an individual cytokine signature after immunization, which can differ markedly from the parent organism. Furthermore, immunization with porins, but not flagellin,

is sufficient to reproduce much of the Th1 and Th17 cytokine profile associated with infection with live Salmonella. This indicates that by carefully selecting the bacterial antigen used as an adjuvant that it is possible to modulate the direction of the immune response induced. These findings are relevant for the rational design of vaccines.

Antibodies generated against bovine CD205 using a bioinformatics approach

Elizabeth Salinas-Estrella[1], Juan Mosqueda[1], Gabriela Aguilar-Tipacamú[1], Patricia Zamorano[1], Valeria Quattrocchi[1]

[1]Universidad Autónoma de Querétaro, Santiago de Querétaro, Mexico
[2]Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina
mvz.elisalinest@gmail.com; joel.mosqueda@uaq.mx; gabtipaca@yahoo.com.mx; zamorano.patricia@inta.gob.ar; quattrocchi.valeria@inta.gob.ar

Keywords: Dendritic Cells, bioinformatics, bovine, CD205, antibodies.

Dendritic cells are the most efficient antigen presenting cells and have an important role recognizing pathogen-associated molecular patterns, thus activating the adaptive immune response. CD205 is a pathogen recognition receptor expressed by dendritic cells and antibodies against this molecule have been used as adjuvants, improving and directing the immune response to vaccine antigens. In this work we generated antibodies to bovine CD205 by using a bioinformatics approach. First, we identified and selected two peptides, which contained predicted B cell epitopes specific of the C-type lectin-like domain of bovine CD205. Synthetic peptides were then used to generate antibodies in rabbits and the specificity of each antiserum was evaluated by indirect ELISA. The antibodies did detect CD205 in tissue sections of bovine lymph nodes, skin and spleen by immunohistochemistry. Afferent Lymph Dendritic Cells (ALDCs) were collected from bovine afferent lymphs and were analyzed by flow citometry with the specific antibodies. We showed that the rabbit serum against bovine CD205 identified a similar percentage of migrating ALDCs than a commercial monoclonal antibody. By using bioinformatics, we developed an accurate, fast and cheap methodology to generate antibodies against the bovine receptor CD205 with detection levels matching those of commercial monoclonal antibodies.

Differential responses to a Bovine respiratory disease challenge based on Bovine Herpes Virus 1 antibody titer status

Paul R. Broadway, Jeff A. Carroll, Nicole Burdick Sanchez

Agricultural Research Service (USDA), Washington D.C., United States
rand.broadway@ars.usda.gov; jeff.carroll@ars.usda.gov; nicole.sanchez@ars.usda.gov

Keywords: Cattle, temperature, immunology, respiratory disease, Bovine herpes virus, Titer

Bovine respiratory disease (BRD) is the most common and costly diseases in the beef cattle industry. However, advancements in vaccination, treatment, and management practices have yielded little to no improvement over the past five decades. Therefore, scientists seek to better understand what factors influence the progression of this disease, and are pursuing alternative ways to reduce and mitigate the negative impacts associated with BRD. The objective of this study was to determine if Bovine Herpes Virus 1 (BHV-1) antibody titer status would be predictive of the physiological and immune responses of beef heifers to a combined viral and bacterial BRD challenge. To accomplish this objective, 64 intact female (heifer) beef calves (~260 kg) were selected and balanced based on body weight, phenotype, and temperament ~30 d after arrival to a commercial feedlot in the panhandle west Texas. All heifers were vaccinated twice with a five-way modified-live respiratory vaccine >30 d before selection. At selection, 32 heifers presented with protective antibody titers to BHV-1 (≥16: Protected: P), and the other 32 presented with no antibody titers to BHV-1 (0; not protected: NP). Heifers were relocated to the USDA-ARS, Livestock Issues Research Unit's Research Complex in Lubbock, TX. Calves were administered 1.0 x 108 PFU of BHV-1 in each nostril via a nasal atomization device (d-3) and were held in covered dirt pens with access to feed ration and water. Additionally, calves were fitted with indwelling vaginal temperature (VT) recording devices set to record vaginal temperature at 5-min intervals for the duration of the study. On d 0, calves were fitted with indwelling jugular catheters and were administered ~2.0 x 107 CFU of log phase Mannheimia Haemolytica (MH) culture intratracheally in 100 ml of Tryptic Soy Broth to ensure adequate passage and lung coating. Following the MH challenge, calves were relocated to bleeding stalls in an environmentally controlled enclosed facility where serum and whole blood were collected every h for 8 h, every 12 h for 72 h, and again on d 7 to evaluate serum biomarkers and hematology, respectively. At 72 h post-MH challenge, jugular catheters were removed and heifers were treated with florfenicol. Nasal lesion scores were observed and recorded on d -3, 0, 3, and 7 relative to the MH challenge. Vaginal temperature patterns varied ($P < 0.05$) in that P heifers exhibited a spike in

VT immediately following the MH challenge (1 h to 12 h) but retuned to baseline within 24 h. Conversely, NP heifers' VT did not spike, but rather declined immediately following the MH challenge, and exhibited a delayed increase where VT was elevated above baseline from 30 h to 72 h. Interestingly, nasal lesion severity was more severe ($P < 0.05$) in P heifers when compared to NP. There were no differences ($P > 0.05$) in red blood cells, hematocrit, hemoglobin, lymphocytes, monocytes, or eosinophils between the treatments. Additionally, there was no difference ($P > 0.05$) in circulating concentrations of cortisol between NP and P heifers. Cortisol remained near basal concentrations following the MH challenge, and cortisol was only influenced by moving heifers through the working facility. Finally, P heifers had increased circulating neutrophils following the MH challenge when compared to NP heifers from 0 to 12 h ($P < 0.05$). In summary, variations exhibited between P and NP heifers in response to a controlled BRD challenge suggest minimal predictive value on the negative impacts of BRD based solely on selection for BHV-1 antibody titers.

Retrospective analysis of relationships between pro-inflammatory, hematology, and metabolic variables, and mortality following a bovine respiratory disease challenge in weaned calves

Nicole C. Burdick Sanchez, Paul R. Broadway, Jeffery A. Carroll

Livestock Issues Research Unit, Agricultural Research Service (USDA), Lubbock, United States
Nicole.Sanchez@ars.usda.gov; Rand.Broadway@ars.usda.gov; Jeff.Carroll@ars.usda.gov

Keywords: Cattle, Mortality, Metabolites, respiratory disease, acute phase response

Respiratory disease is the number one illness affecting the cattle industry, resulting in an estimated $2 billion in losses per year. The Livestock Issues Research Unit has utilized a combined viral-bacterial respiratory challenge model in order to study the pathogenesis of the disease. In a recent study, there was uncharacteristically high mortality in response to the challenge. Therefore, an analysis was conducted to determine whether any of the physiological, immunological, or metabolic parameters measured would be predictive of calf death in response to a controlled respiratory disease challenge. Thirty-one crossbred beef calves [n = 16 castrated males (steers) and 15 intact females (heifers)] were acquired and transported to the Livestock Issues Research Unit's Liberty Farm facility near Lubbock, Texas, USA. Calves were selected based on uniform phenotype characteristics and body weight, and no clinical signs of illness nor treatment history. Additionally, calves were balanced by Bovine Herpesvirus-1 (BHV-1) titers measured 2 weeks prior to the initiation of the study. All calves were previously vaccinated thrice with a combination 5-way respiratory modified-live vaccine without a bactarin in accordance with the farm processing protocol where the calves were acquired. Additionally, calves were fitted with indwelling rectal or vaginal temperature monitoring devices to allow monitoring of body temperature continuously at 5-min intervals prior to relocation to the research facility. The following day, calves were challenged intranasally with 1 x 10^8 PFU of BHV-1 in 1-mL PBS in each nostril using an atomizer. The calves were then allowed to rest in covered outdoor pens for 72 h. The calves were then processed through a working facility where they were fitted with indwelling jugular vein catheters and challenged intratracheally with Mannheimia haemolytica (MH) in log-phase growth at a dose of 1.27 x 10^7 CFU in PBS. Relative to the MH challenge, two whole blood samples were collected at -72 (time of BHV-1 challenge), 0 (time of MH challenge), 2, 4, 6, 8, 12, 24, 36, 48, 60, 72 and 168 h. Serum was isolated from one sample for determination of glucose, non-esterified fatty acids, serum urea nitrogen, interleukin-4 (IL-4), interleukin-6

(IL-6) and interferon-γ (IFN-γ) concentrations. The second blood sample was used to determine complete blood counts (red blood cells, hemoglobin, hematocrit, platelets, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils) in real time using a ProCyte Dx Hematology Analyzer (IDEXX, Westbrook, Maine, USA). Seventy-two hours following administration of MH, catheters and temperature monitoring devices were removed, and all calves were treated with a florfenicol and placed back in the covered outside pens for 4 days. An additional blood sample was collected on d 7 (168 h post-MH challenge) before calves were shipped to a research feedlot approximately 160 km away. Of the 31 calves on study, 10 calves (6 steers and 4 heifers) died in response to the challenge. Three calves died at approximately 48 (1 calf) and 72 h (2 calves) relative to MH challenge. The remaining seven calves died 15 days or more following the MH challenge. To determine what factors may be indicative of calf death, stepwise logistics regression was performed on variables measured (cytokines, metabolites, hematology and body temperature) immediately prior to BHV-1 administration (-72 h) and at the peak temperature response following MH challenge (12 h). Results from the analysis found that of the 17 variables measured, only IL-4 ($P = 0.08$) was considered predictive of calf death prior to BHV-1 administration. Additionally, IL-6 concentration ($P = 0.08$) at the time of peak febrile response was the only variable predictive of subsequent calf death. Further Pearson correlation analysis at the two time points revealed a weak to moderate negative correlation of calf death with IL-4 ($r = -0.38$, $P = 0.03$) and serum urea nitrogen concentrations ($r = -0.29$, $P = 0.11$) with variables measured prior to BHV-1 administration (-72 h). For variables measured at peak febrile response (12 h), platelets ($r = 0.34$, $P = 0.06$), eosinophils ($r = 0.33$, $P = 0.07$) and basophils ($r = 0.37$, $P = 0.04$) were moderately positively correlated with subsequent calf death, while IL-6 ($r = -0.40$, $P = 0.03$) and serum urea nitrogen ($r = -0.29$, $P = 0.11$) were weak to moderately negatively correlated with subsequent calf death. In summary, some variables associated with the acute phase response, as well as serum urea nitrogen concentrations, may be predictive of subsequent calf death. However, these data suggest that there is no single variable that can be measured that would indicate calf death to a combined viral-bacterial respiratory disease challenge in weaned beef calves.

Characterization of cDC1 and cDC2 and the expression of DEC205 on porcine lymphoid tissues

Lucinda Puebla-Clark[1], Héctor Parra-Sánchez[1], Mónica Reséndiz[1], Olivia Valenzuela[2], Jesús Hernández[1]

[1]Center for Research in Food and Development (CIAD), Hermosillo, Mexico
[2]Universidad de Sonora, Hermosillo, Mexico
lucy_pp_c@hotmail.com; parraqh@gmail.com; mresendiz@ciad.mx; valenzuela.o@gmail.com; jhdez@ciad.mx

Keywords: Dendritic Cells, porcine, DEC205, Cdc2, cDC1

Dendritic cells (DCs) are known as the professional antigen presenting cells, and their function is essential for an effective immune response against pathogens. Overall, there are two major types of DCs, the conventional or classical DCs (cDCs) and the plasmacytoid DCs (pDCs); the cDCs are divided into two different subtypes: cDC1 and cDC2. The cDC1 is characterized by promoting a Th1 response, while the cDC2 promotes Th2 and Th17; nevertheless, these cells have not been characterized in porcine lymphoid tissues. These cells express several membrane receptors that help them to carry out their function, a group of these receptors is the C-type lectin receptors (CLRs) that participate in the antigen recognition process. The DEC205 receptor, a very important member of the CLRs family, has the capability to induce and potentiate different immune responses. The DEC205 receptor was characterized in swine, and the expression on porcine blood cDC1 and cDC2 was performed finding that both subtypes were positive for this receptor; however, the expression of DEC205 on lymphoid cDC1 and cDC2 is unknown. Hence, the purpose of this work was to characterize the cDC1 and cDC2 subtypes in different lymphoid tissues and to detect the expression of DEC205 on these cells. The cDC1 and cDC2 subtypes were characterized in tonsil, submaxillary, mesenteric and spleen on 2–4 months old pigs by a four and five-color flow cytometry. The subsets of cDC1 and cDC2 were separated and RNA was extracted and further evaluated for the expression of the FLT3, XCR1 and FCeR1α transcripts by qRT-PCR. The cDC1 subtype was characterized as CD3-CD21-CD163-MHCIIhighCADM1highCD172a-/low, whereas the cDC2 subtype as CD3-CD21-CD163-MHCIIhighCADM1highCD172+. These phenotypes were confirmed by quantitative reverse transcriptase PCR (qRT-PCR) assay on the expression of characteristic transcripts for cDC1 (FLT3 and XCR1), and cDC2 (FLT3 and FCER1α). The tissue with the highest presence of total cDCs was the spleen, and the prevailing subset in all the tissues was cDC1. In all analyzed lymphoid tissues there was DEC205+ cells but with different frequencies. This research is a very important step on the analysis of bona fide porcine DCs, as well as the investigation on the targeting and functionality of the DEC205+ DCs.

Tilmicosin a key regulator of inflamation in bovine mammary gland

Ismael Martínez Cortés[1], Naray Alejandra Acevedo Domínguez[1], Roxana Olguin-Alor[1], Arimelek Cortés Hernández[1], Violeta Alvarez-Jimenez[2], Marcia Campillo Navarro[3], Héctor Salvador Sumano López[3], Lilia Gutiérrez Olvera[3], Daniel Martínez Gómez[4], José Luis Maravillas-Montero[5], Juan J. Loor[6], Eduardo Garcia Zepeda[1], Soldevila Gloria[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Hospital General Naval de Alta Especialidad (HGNAE), Mexico City, Mexico
[3]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[4]Universidad Autónoma Metropolitana, Mexico City, Mexico
[5]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[6]University of Illinois at Urbana–Champaign, Champaign, United States
ismartinezz@hotmail.com; nara_2305@hotmail.com; rolguinalor@gmail.com; arimelekch@hotmail.com; vio_ajd@yahoo.com.mx; marcy265@hotmail.com; sumano@unam.mx; liliago@unam.mx; dmartin@correo.xoc.uam.mx; maravillas@cic.unam.mx; jloor@illinois.edu; garciaze@unam.mx; soldevi@unam.mx

Keywords: Bovine Virus Diarrhea-Mucosal Disease, Mammary Glands, Animal, regulation, inflamation, Tilmicosin

The bovine mammary epithelial cells (MAC) of mammary gland can react against microorganisms by producing high levels of inflammatory cytokines like IL-1β, IL-6 and TNF-α to establish and maintain a pro-inflammatory environment aimed to eliminate the infection. This microenvironment can becomes toxic after the increasing of the reactive oxygen species (ROS) production and mitogen activated protein kinases (MAPK) activation, features that led to the consequently increase of apoptosis rates and the decrease of caseins production in an irreversible manner.

Tilmicosin, a 16c member of the semi-synthetic macrolides; has been used in the treatment of mastitis showing some anti-inflammatory effects. However the mechanism by which this antibiotic can modulate inflammatory processes in mammary gland is still unknown.

The purpose of this work was to evaluate the effect of tilmicosin treatment (TX) in the modulation of mammary immune response after a S. aureus infection and the effect over caseins production in MAC-T cells. To achieve this goal, cells were treated with tilmicosin (10 μg/mL) as follows: pre-treatment for 12 h (Pre-TIL), treatment for 2 h post-infection (Tx-TIL), or control without treatment. Cells were infected with a

bacterial suspension of S. aureus (MOI 100:1) for 2 hours. Flow cytometry, qRT-PCR and immunofluorescence techniques, were used to evaluate these results.

Our data showed that tilmicosin is highly active against S. aureus and the treatment diminishes bacterial infection in MAC-T cells. Tx resulted in a decreased of ROS production and the inhibition in IL-1β, IL-6 and TNF-α secretion. Moreover tilmicosin Tx resulted in 80% less apoptosis. To investigate the molecular mechanism underlying this effect, we performed phosphorylation analysis by flow cytometry and found that Tx stimulated ERK1/2 and decreased p38 phosphorylation, while maintaining a constant casein production.

These results indicate that Tilmicosin can modulate the inflammatory process and diminish the tissue damage produced by S. aureus infection through the regulation of MAPK phosphorylation and ROS production.

Production and development of an ELISA-PPA for the diagnosis of Mycobacterium avium subsp. paratuberculosis in red deer (Cervus elaphus)

Hernán Santiago Hermida[1], Silvia Colavecchia[1], Bárbara Fernández[1], Guillermo Mereb[2], Marcela Martinez Vivot[1], Jorge Suhevic[1], Ana M Jar[1], Silvia Leonor Mundo[1]

[1]Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Buenos Aires, Argentina
[2]Private practice, Santa Rosa, Argentina
hernan.s.hermida@gmail.com; scolave@fvet.uba.ar; barbarafer8@yahoo.com.ar; mereb@ar.inter.net; mvivot@fvet.uba.ar; jsuhevic@fvet.uba.ar; anamjar2@yahoo.com; smundo@fvet.uba.ar

Keywords: Deer, Paratuberculosis, PPA, ELISA, fecal culture

The diagnosis of paratuberculosis is made by identification of Mycobacterium avium subsp. paratuberculosis in the bacteriological culture of feces. However, this technique requires a minimum of 3 months. Therefore, indirect techniques (ELISA) are currently being used to reduce the diagnosis time. Protoplasmic paratuberculosis antigen (PPA) is the antigen recommended by the OIE (World Organisation for Animal Health).

Paratuberculosis affects domestic and wild ruminants, generating great economic losses in animal production. In Argentina, the deer's breeding fields has been increasing in the last years, and along with it, the demand for rapid and accurate diagnosis for this infection.

The aim of this work was the production of a rabbit anti-deer polyclonal antibody and it application into an ELISA-PPA test. For this purpose, two rabbits were immunized subcutaneously with three doses of 1 mg/mL in PBS of deer gamma-globulin obtained by ammonium sulfate precipitation and emulsified with incomplete Freund's adjuvant. Rabbit serum was precipitated, purified with protein A and characterized. Also titer and cross-reactions with other species were evaluated by ELISA. Two different ELISAs were designed using PPA as antigen. ELISA A was performed using the rabbit anti-deer produced and a HRP-conjugated goat anti-rabbit as secondary antibody. In ELISA B an AP-conjugated goat anti-deer IgG commercially available was used. In order to achieve the best conditions for both assays, different dilutions of known positive and negative deer sera, PPA and each antibody were tested. Samples of feces and sera (n=16), nine positive and seven negative, were evaluated; feces by culture and sera by ELISA A and B. Sensitivity, specificity and concordance strength between each ELISA and the fecal culture were analyzed by ROC curves and Kappa index using MedCalcStatistical

Software. Subsequently, sera from a breeding field suspected of being infected with paratuberculosis (n=155) were evaluated by ELISA A and B.

The obtained titer was >256000 and cross-reactions with other ruminant species were detected: cattle, sheep, llama and goat (>64000). ELISA A obtained a greater sensitivity (85.71%) and specificity (88.89%) than ELISA B (77.78% and 57.00%). The concordance strength with fecal culture was "good" k=0.62 ± 0.19 in ELISA A. On the other hand, ELISA B threw a "weak" value k=0.35 ± 0.24. ELISA A also identified a larger number of positive animals in the suspected herd (76.77% vs. 23.23%).

The observed results demonstrated that the anti-deer polyclonal antibody produced could be useful in serological diagnostic tests in deer with high sensitivity and specificity.

In addition, this antibody would allow us to utilize the same reagent in different species which would reduce the cost of the ELISA test.

Development of an indirect elisa based on conserved peptides for the detection of antibodies against Babesia bigemina

Miguel Angel Mercado-Uriostegui[1], Uriel Mauricio Valdéz-Espinoza[2], Juan Alberto Ramos-Aragón[3], Rubén Hernández-Ortíz[3], Juan Mosqueda[1]

[1]Universidad Autónoma de Querétaro, Santiago de Querétaro, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[3]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Jiutepec, Mexico
miguel.mercado.uriostegui@hotmail.com; umvaldez@gmail.com; ros1958k@hotmail.com; hernandez.ruben@inifap.gob.mx; joel.mosqueda@uaq.mx

Keywords: Diagnóstico, Péptidos, B.bigemina, ELISA indirecta, epítopos B

Bovine babesiosis is an intraerythrocytic parasitic disease caused by protozoa of the genus Babesia and it is transmitted by ticks. This disease is by Babesia bovis and Babesia bigemina, and it causes economic losses in cattle in tropical and subtropical areas. It is estimated that around 2 billion cattle wordwide are exposed to babesiosis. Currently the immunodiagnosis for the detection of antibodies against Babesia is made by the indirect immunofluorescence test (IFAT), however, this technique has serious disadvantages, like low sensitivity and specificity in addition to requiring trained staff. On the other hand, there are B-cell epitopes in proteins involved in invasion of host cells with essential biological functionality, which induce antibodies in all infected animals. These antibodies can be used for the serodiagnosis of the disease. The objective of this work was to develop an indirect ELISA based on peptides, containing conserved B-cell epitopes, for the detection of antibodies against Babesia bigemina in sera of naturally infected cattle. The initial selection of B-cell epitopes from membrane proteins of B. bigemina was performed by BLAST (Basic Local Alignment Search Tool) of the NCBI (National Center for Biotechnology Information) to align the sequence of MIC-1 and GP-45 proteins of B. bigemina and select the specific peptides for this species. Subsequently, the ability to identify the presence of antibodies against B. bigemina in sera from cattle in endemic areas was evaluated. For this, 115 sera were used for MIC-1 and 111 for GP-45 respectively and 6 sera (negative controls) of cattle born in a tick-free zone, the sera were evaluated by IFAT. The plates were coated with 10 µg/ml of peptide MIC1A or GP45A diluted in carbonate-bicarbonate buffer pH 9.6 with 100 µl to each well and incubated overnight at 4 °C. Subsequently, the plates were washes three time with PBS Tween 20, pH 7.4 at 0.05%. Blocking was performed with 5% skim milk in PBS, pH 7.4 and incubated for one hour at 37 °C. At the end of the

blocking step, a washing step was repeated. Then, 100 µl per well of each bovine serum diluted 1:50 in PBS pH 7.4 was added per triplicate and the plates were incubated for one hour at 37 °C. The washing was repeated and 100 µl of secondary antibody was added to each well (goat anti-bovine IgG coupled to peroxidase) and the plates were incubated for one hour at 37 °C. Finally the reaction was revealed with 100 µl OPD and hydrogen peroxide and readed with a spectrophotometer after a 20 minutes incubation period at 450 nm wavelength. The cut-off point was determined using the mean of the absorbances of one of the negative sera plus three times the standard deviation. All sera above the cut-off point were considered positive and those that were found below the cut-off point were considered negative. The Indirect ELISA with the MIC-1A peptide showed that 113/116 positive sera were true positives, zero false positives, three were false negatives and six true negatives (control) compared with IFAT. And for the indirect ELISA using the GP-45 peptide A, it showed that 106/111 positive sera were true positives, zero false positives, five false negatives anf six true negatives (control). The diagnostics sensitivity, diagnostics specificity, concordance, positive predictive value and negative predictive value were calculated. All values were obtained by comparing with golden standar (IFAT). The diagnostics sensitivity for the ELISA with MIC-1A peptide was 97.41 %, and the diagnostics specificity was 100 %, the concordance was 97.54 %, with a positive predictive value and negative predictive value of 100 % of for detection of antibodies against B-cell epitopes of MIC-1 protein. The indirect ELISA with the GP-45A peptide reached a diagnostic sensitivity of 95.49 % and the diagnostic specificity of 100 %, a concordance of 95.72 %, a positive predictive value and a negative predictive value of 100 % for detection of antibodies against B-epitopes of GP-45. In conclussion, the indirect ELISA developed in this work is able to detect antibodies in sera of bovines from endemic areas infected naturally. The identification of conserved B-cell epitopes is essential for the development of automated, sensitive and specific diagnostic tests such as indirect ELISA.

Macrophage apoptosis induction by proteins and lipids of Mycobacterium bovis

Itzel Nalleli Jiménez Vázquez[1], Anaid Maciel Rivera[1], Alejandro Benítez Guzmán[1], Hugo Esquivel Solís[2], Clara Espitia Pinzón[1], Cristina Parada Colín[1], Lourdes Arriaga Pizano[3], José Ángel Gutiérrez Pabello[1]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (CIATEJ - CONACYT), Guadalajara, Mexico
[3]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
itzalx1402@hotmail.com; anaid_maciel@hotmail.com; ale.benitezg@gmail.com; hugoesquivels@gmail.com; espitia@biomedicas.unam.mx; cristinaparada@yahoo.com.mx; landapi@hotmail.com; jagp@unam.mx

Keywords: Apoptosis, Mycobacterium bovis, Tuberculosis, immune response, bovine macrophages, protein and lipid fractions

Species of the Mycobacterium tuberculosis complex are able to survive and replicate within the macrophages, some of the mechanisms used by these bacteria are inhibition of the acidification on the phagosome, as well as fusion of the phagolysosome; also they can modulate apoptosis to survive.For the first time our group found that Mycobacterium bovis is capable to induce apoptosis in bovine macrophages and in bystander cells. Moreover, we had proved that both the micobacteria and its protein extract are capable of inducing apoptosis. In order to evaluate the role of different Mycobacterium bovis protein fractions (Culture Filtrate (CFE) and Soluble extract (ES)) and lipids in apoptosis induction we performed a flow cytometry analysis of macrophages incubated in the presence of 3 groups of different protein fractions (ES and CFE each one with three groups) and 19 lipid fractions (100 µg protein and 80 µg lipid/500 000 cells, 24 hrs incubation). Nuclear fragmentation was calculated by TUNEL staining. We found two protein fractions from the ES with a molecular weight between 20–50 kDa and 10–25 kDa (3, 4.17 fold increase each one) with the capacity to induce apoptosis. Also we identify 7 lipid fractions with this ability to induce apoptosis (3.86, 3.79, 2.72, 2.73, 3.69, 2.29, 2.29 fold increase each one). Our results demonstrate both lipids and proteins have the capacity to induce apoptosis; this could help to a better understanding on the interaction between bacteria and the immune response of the host.

Isolation and identification of turkey pox virus and development of an homologous vaccine prototype

José Eduardo Nolasco Esrtrada, José Antonio Quintana López, Laura Cobos Marin, Lucía Eliana Rangel Porta, Liliana Manuela Valdés Vázquez

Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
superman_00pp@hotmail.com; quintanajose98@yahoo.com.mx; laura.cobosmarin@gmail.com; eliana@unam.mx; lilivv22@yahoo.com

Keywords: Turkey, Vaccine, protection, Pox virus, homologous pox virus vaccine

In Mexico turkeys (Meleagris gallopavo) have great social, cultural and economic value, they are considered the second most important species for the national poultry industry. Meleagriculture is developed mainly in backyard systems in rural communities of the country, where there are no well-established health programs, which leaves these animals exposed to many infectious pathogens.

One of the most important diseases of viral origin is the fowlpox. This disease is caused by an Avipoxvirus that produces pustular lesions, weight loss, decreased growth and affects individuals of all ages. In areas where disease is endemic, the best way of prevention is vaccination. However, in Mexico the vaccines used for prevent this disease are made with fowlpoxvirus and used in turkeys, chickens and pigeons.

Previous studies have shown, in different species of birds, that fowlpox homologous vaccination confers better protection than heterologous one. This work compared the protection conferred between a commercial fowl poxvirus vaccine and a turkey poxvirus vaccine.

Turkey poxvirus used to prepare the attenuated active virus vaccine was isolated from scabs of infected turkeys with fowlpox by inoculation into chorioallantoic membranes of 9-day-old chicken embryos.

Viral identification was made by histopathology, polymerase chain reaction and sequencing. Once isolated and identified was adapted in chicken embryo through passages in chorioallantoic membrane and used as a vaccine prototype when it had a titer higher than 104 DIEP50%/mL.

For this trial 3-week-old turkey poults were vaccinated with the elaborated vaccine prototype (n=10), with the commercial vaccine (n=10) and an unvaccinated control

group was maintained (n=10). Animals vaccinated with homologous vaccine achieved 97.7 sera neutralization titer, while those vaccinated with heterologous vaccine achieved 11.7 titer, and it was null for control group.

Finally, animals were challenged with a suspension of turkey pox field virus, which 100% protection was obtained in the group of the vaccine prototype, while in the commercial vaccine group the protection was only 10% and in control group no protection was found.

In conclusion, the use of an homologous pox virus vaccine when working with turkeys, generates neutralizing antibodies and confers a much better protection against infection when compared to the use of commercial vaccines made against the chicken poxvirus.

Development of a multiplex test for diagnosis of goat diseases

Ariadna Verónica Ramos Rodríguez[1], Laura Cobos Marin[1], Alejandro De Las Penas[3], Ana Delia Rodríguez Cortez[1], Héctor Daniel Nájera Rivera[1], Norma Concepción Vázquez Franco[1], Irlanda Judith Siliceo Cantero[1], Aline Hernández Ramírez[1], Liliana Manuela Valdés Vázquez[1], María Grisel Anaya Santillán[1], Efrén Diaz Aparicio[1]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Institute for Scientific and Technological Research, San Luis Potosí, Mexico
arirrbb@gmail.com; laura.cobosmarin@gmail.com; cano@ipicyt.edu.mx; ana_delia_rc@yahoo.com; hdaniel1009@hotmail.com; ncvfvet@gmail.com; irjusica@hotmail.com; ahr6519@hotmail.com; lilianavv22@yahoo.com.mx; anayasantillan@yahoo.com.mx; efredia@yahoo.com

Keywords: Brucellosis, Goat Diseases, Paratuberculosis, Luminex, Arthritis encephalitis

Abstract

Mexican caprine production plays an important role on economy and development of several regions inside the country because of relation between production and sectors alienated of Mexican society. This sector has not enough resources to realize a serological routine diagnostic of diseases most relevant economically important in goats, considered by the Animal´s health advisory technical committee(CONASA); Arthritis encephalitis in goats (AEC), Paratuberculosis (PTB) and Brucellosis as the main diseases for which does not exist any quick and multiple diagnostic test, routinely the use of commercial expensive test that allowed determinate one sickness by time, with higher cost per test, longer test time and making sample´s use less efficient. Projects' objective is the development of a diagnostic system for this 3 sicknesses through Multiplex Luminex system, using a commercial antigen, 2 recombinant proteins and one polysaccharide of Brucella melitensis. With the use of these antigens, we have got a sensitivity of 97.92% and specificity of 95.92% for Brucella melitensis; a sensitivity of 97.78% and 98.89 of specificity for Arthritis Encephalitis and 100% of sensitivity and specificity for Paratuberculosis. This shows that Multiplex Luminex technique is valid and reliable determining those diseases with high diagnostic value.

Introduction: Caprine production in Mexico plays an important role on economy and development of several regions inside the country. It is an activity which has been promoting in recent years and which generates a production of 160,000 tons of milk and 40,000 tons of meat by year.

On 2010; Paratuberculosis, Brucellosis and Caprine Arthritis Encephalitis were marked by CONASA in list of principals diseases that affect goats in the country, and were considered as the sicknesses most important in the productive development for this specie and its difficult diagnosis.

Diagnosis Tests

Mexican official diagnostic tests for these diseases only include monoplex techniques, that meant that only detect a disease by time. For Brucella´s case there are Card test or Rose Bengal that use as antigen strain 1119-3 of B. abortus; and Complement fixation as confirmatory test. Most reliable method for PTB detection is Stool culture and serological diagnostic is realized using indirect ELISA technique. AEC´s diagnostic most useful laboratory tests are Double agar gel immunodiffusion test and ELISA, therefore PCR and RT-PCR are considered complement of serological tests.

Luminex as Diagnostic Test

Monoplex tests have the great disadvantage of use large amounts of sample, also require longer test times , therefore is more expensive and more likely to risk in the stability of the test for the current exposition of repeated cycles of freezing-defrosting. For this reason the multiplex´s technical offered the primary advantage of diagnostic many diseases at the same time, giving the same value to the high sensitivity and specifity.

The technical luminex manifold uses identifiable microspheres by the mixture of Fluorophores interns that allowed the analysis of different agents in only one test since the emission spectrum of each of these microspheres is unique, what makes possible the simultaneous identification of different regions.The assay immunoreactive are anchored to the surface of microspheres and are shown with a fluorescent conjugate is detected in a wavelength other than the issuance of internal fluorescence microsphere. the reading on the equipment is based on flow cytometry , where a needle aspirates the microspheres of the 96-well plate and is subjected on an analysis by means of two lasers: a red laser of 635nm, which simulates the internal region and identifies the specific area of the sphere, denoting an amount the analyte of interest,the result is reported in Medial Fluorescence Intensities

Material, Methods and Results

1.- Native Hapten of Brucella melitensis

It has been demostrate that native hapten (HN) obtained from 16M strain that can differentiate animals vaccinated from infected, is composed mainly of sugars

N-formylperosamine and has a molecular weight of 14.5 kDa. It is an intracellular antigen , which generates immunity only to extend exposure, as is the case of field infection, which produces antibodies against this antigen and not when it is a temporary exposure to the microorganism, as in case of revaccination. HN hapten is obtained from the extraction by means of 3 processes of precipitation and centrifugation of bacterial culture.

Using 50 negative and 50 positive sera it has been obtained a cut point of 288 IMF with a sensitivity of 97.92% and a specificity of 95.92%.

2.-To obtain Caprine Arthritis Encephalitis antigens, it was carried a phylogenetic and bioinformatic analysis of Lentivirus of small ruminants, oligonucleotides were designed with which the genotype and viral subtype were analyzed and identified by PCR in a group of goats from FMVZ- UNAM.

Results obtained through diagnostic PCR demostrated that variety found in Mexico belongs to the LVPR genotype B1,cause of this, it was possible to design and build oligonucleotides that amplified genes that codified for proteins p16-MA, p28-CA, p14-NC and gp38-TM. Proteins were cloned in plasmid pET24 and inserted in the strain BL21-Codon Plus (DE3) -RIL of Escherichia coli to express recombinant proteins induction.

Indirect ELISA gp38

178 sera previously identified by commercial ELISA, 88 were positive and 90 negative to gp38; cut point was an optical density of 0.2458, with a sensitivity of 98.88% and specificity of 96.59%

Luminex p16

Using 90 negative and 90 positive sera it has been obtained a cut point of 1149 IMF with a sensitivity of 97.78% and a specificity of 98.89%.

3. For Paratuberculosis disease, commercial protoplasmic antigen was used, which corresponds to bacterial lysate from Mycobacterium avium strain (Allied monitor laboratory).

Using 11 negative and 11 positive sera it has been obtained a cut point of 623.5 IMF with a sensitivity and specificity of 100%.

Conclusions: The P16 antigens of AEC, PPA-3 of PTB and the native Hapten of BM were recognized by goat sera positive to these diseases by means LUMINEX system was valid and reliable technique as a diagnostic test considering parameters of cut-off point, sensitivity, and specificity.

Atypical granuloma formation by Mycobacterium bovis in young calves

Jacobo Carrisoza Urbina[1], Elizabeth Morales Salinas[1], Mario Adán Bedolla[1], Rogelio Hernandez Pando[2], José Ángel Gutiérrez Pabello[1]

[1]*Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico*
[2]*Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico*
jacobourbina@yahoo.com.mx; moraless@unam.mx; adan_vet@hotmail.com; rhdezpando@hotmail.com; jagp@unam.mx

Keywords: Mycobacterium bovis, calcification, natural infection, Atypical granulomas, bovine tuberculosis.

Mycobacterium bovis (*M. bovis*) is the etiological agent of bovine tuberculosis which is a chronic inflammatory disease characterized by production of granulomatous lesions (Domingo et al., 2014). Several studies have been focused on characterizing granulomas in experimentally infected cattle, which has been useful to understand the pathogenesis of the infection and to allow assessment of the protection of prototype vaccines (Aranday-Cortes et al., 2013; Buddle et al., 2011; Majoor et al., 2011; Waters et al., 2012; Wangoo et al., 2005). Nevertheless, few studies have reported macroscopic and microscopic characteristics of these lesions in cattle naturally infected by *M. bovis* (Menin et al., 2013). Therefore, in the present study granulomas from 32 Holstein Friesian cattle of a dairy area in central Mexico were studied. In this opportunity sampling 46.8% (15/32) cattle were less than 4 months of age and 53.2% (17/32) were more than one year old. Macroscopically, 100% (32/32) of the animals included, developed lesions suggestive of tuberculosis in mediastinal lymph nodes and 50% (16/32) of lesions were identified in lungs, from these tissues 1143 granulomas were analysed microscopically, of which 34.6% (396/1143) were from 1-year-old cattle or older and were classified according to the methodology of Wangoo et al. 2005, resulting in 34.3% (136/396) at stage IV and 29.0% (115/396) were stage I, frequently were satellites from stages III and IV. Surprisingly, lesions in cattle under 4 months of age showed an unusual pattern that interfered with classification. These granulomas features include large areas of necrosis extending in most of the affected organ, central calcification, absence of connective tissue capsule, an average of 1.4 giant cells per lesion and many positive acid-alcohol resistant bacillus, the adult animals had the highest percentage of lesions without bacilli with 47.9% and only 7.4% of this type of lesions was seen in the group of animals less than four months old (Figure 1). The presence of *M. bovis* was confirmed in 84.3% (27/32) of the cases analyzed by PCR. These observations suggest that cattle under 4 months of age are unable to control naturally *M. bovis* infection. This knowledge can be useful in understanding natural host resistance to mycobacterial infections.

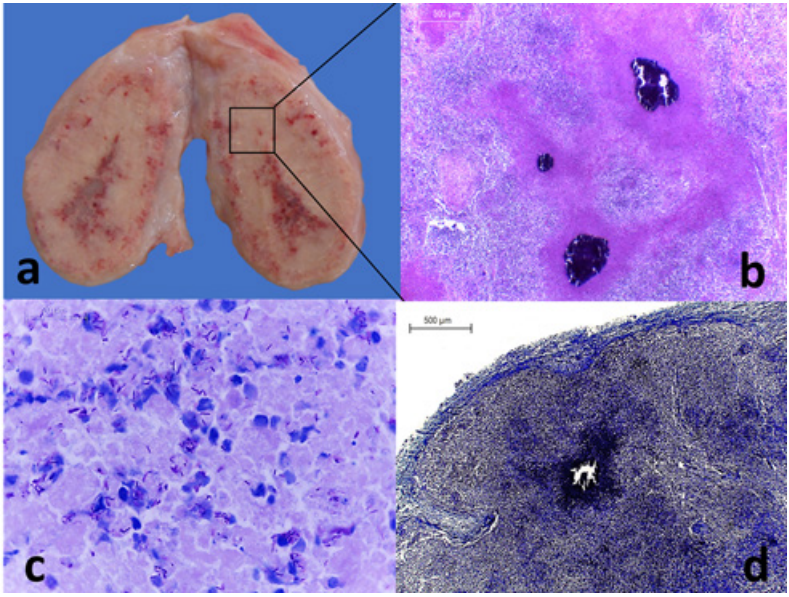


FIGURE 1: Atypical granulomatous lymphadenitis in cattle under four months of age caused by *Mycobacterium bovis*. (a) Mediastinal lymph node of a 2-month-old bovine with extensive areas of caseous necrosis at the cut. (b) H & E, 40x lymph node with abundant areas of necrosis and calcification without defined borders. (c) Zielh Nelseen stain, 1000x lymph node with abundant extracellular alcohol-resistant acid bacilli. (d) Masson's Trichromy stain, 40x calcified granulomatous lesion without connective tissue capsule.

REFERENCES

Aranday-Cortes, E., Bull, N. C., Villarreal-Ramos, B., Gough, J., Hicks, D., Ortiz-Peláez, A., et al. (2013). Upregulation of IL-17A, CXCL9 and CXCL10 in early-stage granulomas induced by *Mycobacterium bovis* in cattle. *Transbound. Emerg. Dis.* 60, 525–37. doi:10.1111/j.1865-1682.2012.01370.x.

Buddle, B. M., Wedlock, D. N., Denis, M., Vordermeier, H. M., and Hewinson, R. G. (2011). Update on vaccination of cattle and wildlife populations against tuberculosis. *Vet. Microbiol.* 151, 14–22. doi:10.1016/j.vetmic.2011.02.021.

Domingo, M., Vidal, E., and Marco, A. (2014). Pathology of bovine tuberculosis. *Res. Vet. Sci.* 97 Suppl, S20-9. doi:10.1016/j.rvsc.2014.03.017.

Majoor, C. J., Magis-Escurra, C., van Ingen, J., Boeree, M. J., and van Soolingen, D. (2011). Epidemiology of Mycobacterium bovis disease in humans, The Netherlands, 1993–2007. *Emerg. Infect. Dis.* 17, 457–63. doi:10.3201/eid1703.101111.

Menin, Á., Fleith, R., Reck, C., Marlow, M., Fernandes, P., Pilati, C., et al. (2013). Asymptomatic cattle naturally infected with *Mycobacterium bovis* present exacerbated tissue pathology and bacterial dissemination. *PLoS One* 8, e53884. doi:10.1371/journal.pone.0053884.

Tsairidou, S., Woolliams, J. A., Allen, A. R., Skuce, R. A., McBride, S. H., Wright, D. M., et al. (2014). Genomic prediction for tuberculosis resistance in dairy cattle. *PLoS One* 9, e96728. doi:10.1371/journal.pone.0096728.

Wangoo, A., Johnson, L., Gough, J., Ackbar, R., Inglut, S., Hicks, D., et al. (2005). Advanced granulomatous lesions in *Mycobacterium bovis*-infected cattle are associated with increased expression of type I procollagen, gammadelta (WC1+) T cells and CD 68+ cells. *J. Comp. Pathol.* 133, 223–34. doi:10.1016/j.jcpa.2005.05.001.

Validation of two indirect enzyme-linked immunosorbent assays for the diagnosis of canine brucelosis

Victor Palacios Rodríguez, Beatriz Arellano Reynoso, Aldo Morales Aguilar, Alejandro Benítez Guzman, Francisco Suárez Güemes

Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México Mexico

mvzpalaciosr@gmail.com; arerey@yahoo.com; over.the.hallways@outlook.com; ale.benitezg@gmail.com; fsg@servidor.unam.mx

Keywords: Brucella canis, GroEL, Indirect ELISA, Canine brucellosis, Diagnostic test validation

Canine brucellosis is an infectious disease of the reproductive system caused by Brucella canis. Its importance lies in the compromise of fertility in bitches used for reproduction and, mainly, in its potential to cause disease in humans representing an important public health problem [1]; in spite of this, the only approved tests for the diagnosis of this disease are the Rapid Slide Agglutination Test (RSAT) and the blood culture in specialized laboratories [2], giving to the development of useful diagnostic tests an important place in the scientific research. The goal of this work is to develop and validate two indirect enzyme-linked immunosorbent assays (iELISA) for the diagnosis of canine brucellosis.

Two iELISA tests were standardized, one using crude antigen (CA) of Brucella canis strain RM6/66, and another one using the 60 kDa chaperonine GroEL, identified as immunodominant.

The first test was developed using 96-well polystirene Mid-binding plates (Sarstedt AG & Co. Nümbrecht, Germany), there were added 240 ng of CA diluted in 100 µL per well of PBS, it was shaken at 100 rpm, 5 min and incubated at 37º C, 17 h +/- 1 h. It was washed three times using Tween 20 (SIGMA, Darmstadt, Germany) diluted at 0.1% in PBS. 100 µL per well of bovine serum albumin (BSA) diluted at 1% in PBS were added, it was incubated at 37º C for 60 min. It was washed three times using Tween 20 diluted at 0.1% in PBS. The sera from dogs used as positive and negative controls was diluted at 1:250 in PBS and then 100 µL of the solution were added per well, it was incubated at 37º C for 60 min. It was washed three times using Tween 20 diluted at 0.1% in PBS. Then, a 1:2000 solution of rabbit-produced anti-canine IgG antibody conjugated with horseradish peroxidase (SIGMA, Darmstadt, Germany) in PBS was prepared, adding 100 µL of this solution per well and incubating at 37ª C for

60 min. It was washed three times using Tween 20 diluted at 0.1% in PBS. Finally, 100 µL of ABTS (SIGMA, Darmstadt, Germany) were added in darkness conditions, and then the plate was covered with aluminum foil and incubated in orbital shaking (100 rpm) at room temperature for 25 min. It was read in an absorbance reader (Biotek Instruments Inc. Winooski, VT, USA) at a wavelength of 405 nm using Gen5® software.

The second test were developed using 96-well MaxiSorp™ (Merck, Darmstadt, Germany) plates, adding 0.01 µg of GroEL diluted in 100 µL of carbonate buffered solution (0.05 M; pH 9.6) per-well, shaking it at 100 rpm, 5 min and then incubating at 4º C, 17 +/- 1 h. It was washed four times using Tween 20 diluted at 0.5% in saline solution (1.5 M; pH 7). Then, 100 µL of a 1:1000 dilution of the control sera in 1% PBS + BSA solution was added per-well, incubating it 2 h at 28º C. It was washed four times using Tween 20 diluted at 0.5% in saline solution. A 1:5000 dilution of rabbit-produced anti-canine IgG antibody conjugated with horseradish peroxidase diluted in PBS was prepared, adding 100 µL of this solution per-well and incubating it 2 h at 28º C. It was washed four times using Tween 20 diluted at 0.5% in saline solution. Finally, 100 µL of ABTS were added in darkness conditions, then the plate was covered with aluminum foil and incubated in orbital shaking (100 rpm) at room temperature for 25 min. It was read in an absorbance reader at a wavelength of 405 nm using Gen5® software.

A cut-off point was determined for each test according to the methodology proposed by Frey et. al. [3] as well as the sensibility and specificity using positive and negative dog sera. The determination of robustness was made changing the temperature (37 +/- 5º C for the CA test; 28 +/- 5º C for the GroEL test) and the incubation time (60 +/- 5 min for the CA test; 120 +/- 5 min for the GroEL test); the results were analyzed by an ANOVA test. To determine the repeatability of the test the variation coefficient (%CV) was calculated for independent (5 test in different days) and dependent (a quintuplicated test in the same plate) tests. The selectivity was evaluated using sera from dogs infected with Mycobacterium spp., Leptospira interrogans and Salmonella spp. [4]

The CA iELISA had a cut-off point of 0.98 in optic density (OD), it has a sensibility and specificity of 100% (70%-100% CI95), a %CV < 15% for dependent and independent determinations. There are not difference within the standard test and the tests with variations in time and temperature ($p < 0.05$), however, the sera from dogs infected with L. interrogans and Mycobacterium spp react positively with the CA test.

The results indicate that the CA iELISA is useful for the diagnosis of canine brucellosis [4], nonetheless, we suggest to the clinician to evaluate the patient as a whole, preventing a dismiss of the correct diagnosis and putting special attention on leptospirosis and tuberculosis as these illnesses react with the test [5; 6]. In a future we are going to evaluate the GroEL iELISA to define its utility in the canine brucellosis diagnosis.

REFERENCES

[1] M.X. Byndloss, and R.M. Tsolis, Brucella spp. Virulence Factors and Immunity. Annual review of animal biosciences 4 (2016) 111-27.

[2] F.F. Badakhsh, L.E. Carmichael, and J.A. Douglass, Improved rapid slide agglutination test for presumptive diagnosis of canine brucellosis. J Clin Microbiol 15 (1982) 286-9.

[3] A. Frey, J. Di Canzio, and D. Zurakowski, A statistically defined endpoint titer determination method for immunoassays. J Immunol Methods 221 (1998) 35-41.

[4] U. Andreasson, A. Perret-Liaudet, L.J. van Waalwijk van Doorn, K. Blennow, D. Chiasserini, S. Engelborghs, T. Fladby, S. Genc, N. Kruse, H.B. Kuiperij, L. Kulic, P. Lewczuk, B. Mollenhauer, B. Mroczko, L. Parnetti, E. Vanmechelen, M.M. Verbeek, B. Winblad, H. Zetterberg, M. Koel-Simmelink, and C.E. Teunissen, A Practical Guide to Immunoassay Method Validation. Frontiers in neurology 6 (2015) 179.

[5] S. Barandiaran, M. Martinez Vivot, E. Falzoni, M.J. Marfil, G. Perez Tort, P. Rovatti, M. Fernandez, R. Iachini, F. Satek, A. Duchene, and M.J. Zumarraga, Mycobacterioses in dogs and cats from Buenos Aires, Argentina. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 29 (2017) 729-732.

[6] W. Wang, X. Gao, M. Guo, W. Zhang, X. Song, T. Wang, Z. Zhang, H. Jiang, Y. Cao, and N. Zhang, Leptospira interrogans induces uterine inflammatory responses and abnormal expression of extracellular matrix proteins in dogs. Microbial pathogenesis 75 (2014) 1-6.

Aedes aegypti antiviral adaptive response against DENV-2

Javier Serrato

Instituto Nacional de Salud Pública, Cuernavaca Mexico
javierserrato@gmail.com

Keywords: priming, Aedes aegypti, antiviral, Memory response, DNA synthesis., DENV-2

Priming is the conceptual term defining memory phenomenon in innate immune response. Numerous examples of enhanced secondary immune response have been described in diverse taxa of invertebrates; which naturally lacks memory response. In mosquitoes, a previous non-lethal challenge with some specific pathogens modify their immune response against the same microorganism; developing an improved antimicrobial reaction. In this work, we explore the ability of Aedes aegypti to mount a higher antiviral response upon a second oral DENV challenge. When previously challenged with inactive virus, we observed that the posterior infection showed a diminished number of DENV infectious particles in midguts and carcasses. In challenged tissues, we detected higher de novo midgut DNA synthesis than control group, as determined by DNA incorporation of 5-bromo-2-deoxyuridine. We demonstrated that inactive DENV particle are capable to induce DNA synthesis levels comparable to infective DENV. We considered the Drosophila melanogaster hindsight and Delta-Notch mosquitoes orthologues as potential de novo DNA synthesis pathway components (as observed in fly oocyte development and midgut tissue renewal). We showed that Aedes aegypti hindsight transcript relative expression levels were higher than control during DENV infection and inactive DENV particle alimentation. Also, Aedes aegypti second challenge with active DENV induced higher hindsight, Delta and Notch transcriptions in the primed mosquitoes (compared with the primary infection levels). Considering that the mosquito de novo DNA synthesis is concomitant to viral particle reduction, this finding opens a new perspective on the mechanisms underlying the vector antiviral immune response and the effector molecules involved.

Aedes aegypti midgut DNA synthesis de novo is an essential antiviral strategy against dengue virus serotype 2 replication

Javier Serrato

Instituto Nacional de Salud Pública, Cuernavaca, Mexico
javierserrato@gmail.com

Keywords: denv, Aedes aegypti, delta, antiviral response, hindsight, DNA endoreplication, Notch.

Aedes aegypti is the main vector of Dengue Virus, carrying the virus during the whole mosquito life post-infection. Few mosquito fitness costs have been associated to the virus infection, thereby allowing for a swift dissemination. In order to diminish mosquitoes population, public health agency use persistent chemicals with environmental impact for disease control. Most countries barely use biological controls, if at all. With the purpose of developing novel Dengue control strategies, a detailed understanding of the virus-vector interactions is urgently needed. Damage induced (through tissue injury or bacterial invasion) DNA duplication (endoreplication) has been described in insects during epithelial cells renewal. Here, we delved into the mosquito midgut tissue ability to synthesize DNA de novo; postulating that Dengue virus infection could trigger a protective endoreplication mechanism in some mosquito cells. We hypothesized that the Aedes aegypti orthologue of the Drosophila melanogaster hindsight gene (not previously annotated in Aedes aegypti transcriptome/genome) is part of the Delta-Notch pathway. The activation of this transcriptional cascade leads to genomic DNA endoreplication. The amplification of the genomic copies of specific genes ultimately limits the viral spreading during infection. Conversely, inhibiting DNA synthesis capacity, hence endoreplication, leads to a higher viral replication.

Design and application of the new Immunology program in the context of the Cuban medical education area

Irma Gudelia Vega García[1,2], Mayppe González Jardines[3], Marta Pernas Gómez[3], Alina Josefina Alerm González[1,2], Victoria E González Ramírez[2], Martha L Paradoa Pérez[2], Miriam SJB Lastre González[2], Laura María Reyes Díaz[2], Oliver Pérez[1,2]

[1]Escuela Latinoamericana de Medicina, La Habana, Cuba
[2]Instituto de Ciencias Básicas y Preclínicas Victoria de Girón, Universidad de Ciencias Médicas de la Habana, La Habana, Cuba
[3]Universidad de Ciencias Médicas de la Habana, Havana, Cuba
irmav@giron.sld.cu; gmayppe@yahoo.es; mpernas@infomed.sld.cu; alina.alerm@infomed.sld.cu; victoria.gonzalez@infomed.sld.cu; mparadoa@infomed.sld.cu; oliver.perez@infomed.sld.cu; lreyes@giron.sld.cu; oliverperez@giron.sld.cu

Keywords: Medical Records, Medicine, Teaching, immunology, Medical students

Background: In the current century the superior education constitutes an important tool with which a country can promote its development and to strengthen its national identity and self-determination. Then, it is necessary to consider who should receive benefits from de superior education transformation process. In addition, it is necessary to consider the final functions of the professional, the social need, the social world, and the context to determinate the objectives and knowledge in school programs1,2. In medical education the basic biomedical sciences, particularly Immunology, give the conceptual basis to the curriculum of Medicine and have an important relevance to medical students, to form de basis for a lifetime about de immune system and its potential for use in improving the human condition. The growing amount of information considered essential in basic and clinical disciplines obligates to integrate their knowledge and to advance to a new curricular program. Teaching guidelines and the medical exercise must adequate to the health requirements of the population needs and country politics, taking in consideration the new tendencies in medical education. The problems of health in the current world context need a better understanding of the human immune system and its role in health and disease. Consequently, it is important that medical students see the relevance of studies of immune system in the health human been in order to integrate and to relate with structural and functional change in clinical problems and its psychosocial and ambient components.

Aim: To analyze the contribution of immunology program for medical curriculum organization in correspondence with the medical social needs and current world and Cuban context.

Results: It was performed the new program and it was introduced since the present course (2017–2018) in all forting faculty of medicine in Cuba. It includes: 1, Basic immunology in the health human introduced in a subject with the studies about blood at third semester of medical career (Bood and Immune system with 60 hours). It is a subject in the discipline Biological Bases of Medicine (BBM). Each subjects in BBM where organized taking in consideration the different material levels (molecules, cells, tissues, organs, system, people, and society), the relation between structure and function and phenotypic expression of the human been at their environment3,4,5; 2, Vaccinology course in the Prevention subject at third semester with 20 hours; and 3, Immunopathology in the Pathologic Anatomy subject at fourth semester with 20 hours which is been maintained. The association of immunology with other basic biomedical and clinic subjects, particularly with General Medicine as major discipline, was also included. These permit the integration between their knowledge. The precedencies of other basic biomedical or clinical subjects have been considered in each semester. In addition, optative courses for the other semesters of the career were included. The methodological approaches change, mainly focuses in the structure-function behavior and the basic-clinical interactions. Immunology in the health human ("Inmunologìa en el humano sano") is the title of a new Cuban Immunology putting ad disposition of all student8. It is the basic bibliography and its format is in correspondence with the program of Blood and Immune System subject. Another book for Vaccinology course is been written. New opportunities have been developing for specialty and doctoral formation. The production of didactic materials for practice teaching within undergraduate and postgraduate training tribute to specialty thesis. The didactic strategy tribute to doctoral work.

Conclusions: The new immunology program for undergraduate medical career in Cuba was started at 2017–2018 course. A new Immunology book was published and other is been written like basic bibliographies. These didactic strategies tributes to specialty and doctoral formations in Cuba.

NS1 dengue virus protein affects the arboviral infection in insect cells

Luis Antonio. Alonso-Palomares[1], José Esteban Muñoz-Medina[2], Jorge Isamel Castañeda-Sanchez[3], Ma. Isabel Salazar-Sánchez[1], J. Humberto Lanz-Mendoza[2]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Universidad Autónoma Metropolitana, Mexico City, Mexico
[4]Instituto Nacional de Salud Pública (INSP), Cuernavaca, Mexico
luis_alp88@hotmail.com; Eban10@hotmail.com; jordyfilm@yahoo.com.mx; isalazarsan@yahoo.com; humberto@insp.mnx

Keywords: Chikungunya virus, Dengue, NS1 protein, Inmunology, Mosqutioes

Luis Antonio Alonso-Palomares 1, José Esteban Muñoz-Medina 2, Jorge I. Castañeda-Sánchez3, Humberto Lanz-Mendoza 4, Ma Isabel Salazar 1*
*1Escuela Nacional de Ciencias Biológicas -IPN, México city, Mexico; 2Instituto Mexicano del Seguro Social – Raza, Mexico city, Mexico; Universidad Autónma Metropolitana-Xochimilco, Ciudad de México; 4Instituto Nacional de Salud Pública - CISEI, Cuernavaca, Mor. Mexico. *Email: luis_alp88@hotmail.com*

Introduction: The mosquito Aedes aegypti is the most important vector for transmission of different arboviruses, which include dengue (DENV) and chikungunya virus (CHIKV). Both of these viruses coexist in the Americas and have originated important outbreaks, moreove coinfections with both arbovirus have been reported. Innate immune response in mosquitoes involves different signalling pathways, among these are Toll, IMD, Jak-stat and iRNA pathways. An additional important element in natural infections with DENV is the NS1 viral protein, which effect for virus replication or for the mosquito immune response remains undetermined.

Objective: Determine the effect of NS1 viral protein on the immune response and on virus replication in insect cells coinfected with DENV and CHIKV.

Materials and Methods: We use Aag-2 mosquito cell line preatreated with recombinant NS1 protein, these were infected with either DENV-2, CHIKV or both. We used qRT-PCR to detect the viral genome and to establish replication levels. Also, we examined expression of some molecules that participate in immune pathways such as REL1A,

Cactus, defensin C, (Toll pathway) STAT and PIAS (jak-stat pathway). We examined the NS1 protein localization in Aag-2 cell by confocal microscopy.

Results and Conclusions: The NS1 protein from DENV induced significant changes in the mRNA expression levels for REL1A, Cactus, STAT, and defensin C in the Aag-2 mosquito cells. We observed NS1 protein by immunofluorescence in the Aag-2 cells near endoplasmic reticulum (RE), where coincidentaly virus replication takes place. NS1 protein clearly shorthened DENV replication time in these cells. Strinkingly, this phenomenon allow DENV surpassed CHIKV in replication levels in coinfected cells. Thus, NS1 protein from DENV not only modulates immune response in mosquitoes cells but, also affects virus replication rate conferring an apparent advantage for DENV infectivity. This is a first report showing that NS1 virus protein affects immune response in mosquito cells.

Endoreplication as a mechanism for immunological priming in the mosquito Anopheles albimanus against Plasmodium berghei

Krystal Maya Maldonado[1], Fidel De la Cruz Hernández-Hernández[1], Humberto Lanz-Mendoza[2]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
[2]Instituto Nacional de Salud Pública (INSP), Cuernavaca, Mexico
kmaya@cinvestav.mx; cruzcruz@cinvestav.mx; humberto@insp.mx

Keywords: Immunity, Innate, Plasmodium berghei, priming, Mosquitoes, Anopheles albimanus

Malaria is a disease that affects more than two hundred million people worldwide and each year die between one and two million by this problem. This disease is caused by parasites of the genus Plasmodium, which are transmitted to humans by the bite of female mosquitoes Anopheles. In mosquitos the midgut is one of the organs that plays a very important role in limiting the development of Plasmodium, this is due to the immune response that triggers the gut of the mosquito against the development of the parasite. Interestingly, in invertebrates a phenomenon known as "immunological priming" has been described, defined as the ability to acquire a protective (adaptive) response to a pathogen due to previous exposure of the same organism. In our group an adaptive response (immunological priming) has been reported in the An. albimanus mosquito in which the midgut plays a very important role. So far the mechanism by which this type of immune response that resembles the adaptive immune response of vertebrates is unknown. In this case, invertebrates do not have a lymphocytic cell population capable of responding classically (clonal selection), nor has cell proliferation been reported, which would represent a very strong energy expenditure, nor is there information about the mechanisms involved in the synthesis of the defense molecules that unfold in this response. However in An. albimanus, DNA synthesis has been detected against a challenge with different organisms in different tissues that play a central role in the insect's immune response. This DNA synthesis could be due to an endoreplication process. In nature, many organisms carry out an endoreplication process in order to supply a massive molecule synthesis in a given situation. In D. melanogaster, the endoreplication originates an amplification of specific DNA regions in which genes are placed in demand of important situations such as oogenesis and that need to be produced massively. In addition to this and considering the evidence of DNA synthesis in tissues such as the midgut that shows an important immune response activity against Plasmodium and other pathogens, it is possible to suggest that the production of immune molecules involves the expansion of genes by endoreplication.

Characterization of pericardial cells immune response in the malaria vector Anopheles albimanus

Salvador Hernandez-Martinez[1,2], Victor MJ Cardoso-Jaime[1], Karen E Gutierrez-Alvarez[1], Víctor K Tsutsumi[2]

[1]Instituto Nacional de Salud Pública (INSP), Cuernavaca, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
shernandez@insp.mx; vcardosoj@cinvestav.mx; karenes1902@gmail.com; vtsutsu@cinvestav.mx

Keywords: Anopheles albimanus, lysozyme, Pericardial cells, Mosquito immunity, Mosquito heart

Insects have efficient innate immune mechanisms that allow them to recognize and neutralize pathogens. The identification of organ and tissues involved in these mechanisms is essential for the development of control strategies in vector-borne diseases. The fat body and the hemocytes are the most studied tissues in terms of their immune response. The insect heart contains accessory cells called pericardial cells (PCs) or nephrocytes, with functions of filtration and recycling of molecules from the hemolymph. Recently, several studies have demonstrated the presence of response immune markers in mosquito PCs (STAT PCs; Sp22D, TEP-I and SRPN10), suggesting their possible involvement in the defense mechanisms. Currently, we are studying the response of PCs of Anopheles albimanus against different pathogens. The bioinformatics analysis on the transcriptome of hearts of An. albimanus (using the recent databases of 16 anopheline genomes), showed that the GNBP4B gene was found over-expressed in samples treated with zimosan. In the control library were expressed transcripts from genes that encode the kinase PLL1 and ECSIT (TOLL pathway). On the contrary, library from zymosan challenged samples, were expressed MYD88 and TOLLIP genes. In the IMD pathway, genes IMD and FADD were repressed in samples challenged with zymosan, while IKK1 was expressed only in the control. In addition, were identified 6 Serpins, including SRPN10. An. albimanus genome contains 3 sequences that can encode cecropins (ECSC, CECC, CECD). In the transcriptome, CECA was expressed exclusively in hearts challenged with zymosan; and the CECD was found over-expressed during the challenge with zimosan. In An. gambiae, CECA has been associated with the immune response against Plasmodium. For the design of oligonucleotides was used the PerlPrimer software and sequences were optimized for the conditions of qPCR. As a first step, 4 reference genes were evaluated to be used as internal control, in hart samples from different treatments. The results indicate that the best reference gene is GAPDH, given that did not show significant changes during the time and the different treatments; followed by the ribosomal genes RpL27

and RpS7. The kinetics of immune response genes expression, we used three groups of 20 mosquitoes (untreated, inoculated with RPMI, and inoculated with zymosan). Hearts cDNA was generated. The expression of CECA, lysozyme type C-1, SRPN10, GAPDH, RpS7 was evaluated. The ECSC and lysozyme C-1, showed overexpression at 6 h post-inoculation with RPMI and zymosan, which indicates that the CECA and lysozyme may be associated with damage in An. albimanus. However, at 12 h lysozyme showed a significant increase. Evaluations by qPCR using total RNA from mosquitoes. First analysis showed a fusion curve with a single peak, indicating that the conditions of the reaction are reliable, since there are no secondary products. We started with the analysis and characterization of lysozyme. In An. gambiae are described 8 genes for lysozyme, but only lysozyme type 1 has been associated to immune response. In the An. albimanus genome are present 3 lysozymes (LYS7, LYS1, LYS6), and in the transcriptome LYS1 was the only identified with a high percentage of identity with the LYS1 of An. gambiae. Then, primers were design using sequences obtained from the VectorBase database and the Primer-BLAST program. A local alignment using the BLASTn between the sense and reverse primers was performed to lysozyme gene. For the design of primers was taken first exon to the sense sequence and a sequence of the second exon to the anti-sense. A gradient-PCR was performed with different temperatures of alignment, and then an endpoint PCR with following conditions: 94°C/3 min, 40 cycles at 94°C/40s, 61°C/30s, 72°C/1 min and an elongation temperature of 72°C/10 min. The amplification product size for lysozyme was the expected. With these conditions, currently we are evaluating the lysozyme expression by qPCR. Finally, the presence of lysozyme in mosquito full extract was evaluated by Western-blot using an antibody anti-lysozyme C from chicken egg. There was a recognition of lysozyme in extracts of mosquitoes An. albimanus and Ae. aegypti with molecular weight close to the 15.4 kDa and 15.9 kDa respectively. Currently we are evaluating the lysozyme changes in mosquito heart samples under different immune challenges. The continuity of these studies is essential to identify and validate the expression of genes related to immunity in the PCs. The results provide new knowledge of the immune mechanisms of diseases vectors.

Salmonella-mediated anti-melanoma effect is dependent on caspase-1 activation

Amy Mónaco, Lucia Yim, Jose Alejandro Chabalgoity, María Moreno

Institute of Hygiene, Faculty of Medicine, University of the Republic, Montevideo, Uruguay
amymonpat@gmail.com; lyim@higiene.edu.uy; jachabal@higiene.edu.uy; mmoreno@higiene.edu.uy

Keywords: Melanoma, Salmonella, Inflammasome, caspase-1, Bacterial immunotherapy

It is widely known that Salmonella is able to invade and replicate within host cells, including epithelial cells and macrophages. Salmonella invasion and subsequent intracellular replication is facilitated by two different type III secretion systems (T3SS) encoded by genes of Salmonella pathogenicity islands 1 and 2, respectively (SPI-1 and SPI-2). Salmonella can also invade tumor cells, and mediate intrinsic tumor cell killing. This ability has been exploited as anti-cancer therapy. Nevertheless, the mechanisms underlying this effect still remain unclear. In this work, we deepen into the activation of the inflammasome complex mediated by Salmonella invasion, and its role in the anti-tumoral effect in a murine melanoma model.

Although the kinetics of entry and survival of Salmonella in macrophages have been widely described, little is known regarding this mechanism in tumor cells. Using different Salmonella strains expressing GFP under the control of SPI-1 or SPI-2 promoters, we found that Salmonella invasion of B16F1 melanoma cells occurs at very early times (less than 30 minutes), while the mechanisms of intracellular survival and replication induced by SPI-2 T3SS take hours to be activated. We therefore infer that Salmonella enters the tumor cell through the mechanisms classically described for phagocytic cells. Once inside the cell, inflammatory pathways are activated, leading to the activation of caspase-1. Using FLICA 660 reactive we found that this caspase-1 activation only occurs when Salmonella SPI-2 T3SS has been activated. Moreover, we found that approximately 50% of tumor cells exhibited caspase-1 activation, even though they have not been invaded by Salmonella. The mechanism underlying inflammasome activation independent of the presence of intracellular Salmonella could be attributed to immunogenic cell death induced by Salmonella in neighboring cells, but clearly more studies remain to be done.

In vivo, activation of the inflammasome is essential for Salmonella to exert its antitumor effect, since caspase-1 knock out (casp-1 ko) mice do not show any improvement after treatment with intratumoral Salmonella while Salmonella-treated littermate (wild-type, wt) mice exhibit tumor growth retardation and prolonged survival. We particularly analyzed the recruitment of neutrophils and macrophages to the tumor 24 hours after

treatment with Salmonella, both in wt and casp-1 ko mice. We found that casp-1 ko mice showed an abrogation in Salmonella-induced neutrophils and macrophages tumor infiltration in comparison with wt mice. Additionally, in wt mice, Salmonella treatment elicits caspase-1 activation in those tumor infiltrating neutrophils ($p=0.0005$). The tumoral levels of active IL1beta in these mice were evaluated by ELISA, with a significant increase in the levels of mature protein found only in the wt mice. In turn, IL18 levels remain unchanged.

Interestingly, when comparing infiltration of distinct lymphocyte populations (CD8+, CD4+ and NK T cells or B cells) between wt and ko mice, no remarkable differences were detected, both in TDLN and tumor.

Taken together our results suggest that upon Salmonella invasion of tumoral cells, inflammasome is activated both in tumoral and immune cells. This activation leads to an increase in caspase-1 activation that is required for Salmonella to exert its anti-tumoral effect.

Orally administered recombinant Taenia solium calreticulin ameliorates experimental colitis

Bernardo Oldak[1], Mayra Cruz-Rivera[2], Estefanía Grostieta[2], Suemy Vega[2], Job Rojas[2], Ana Flisser[2], Fela Mendlovic[1,2]

[1]Universidad Anáhuac México Norte, Huixquilucan de Degollado, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
bernardo.oldak@anahuac.mx; mayracr@yahoo.com; estefigros7@gmail.com; suemyvega@ciencias.unam.mx; jobisaacrl@ciencias.unam.mx; flisser@unam.mx; fmendlo@yahoo.com

Keywords: Calreticulin, Dextran Sulfate, Immunomodulation, Inflammatory Bowel Diseases, Taenia solium

Background: Immunological-induced pathologies, including inflammatory bowel disease, have been rising in developed countries and in developing countries like Mexico, it is expected to have a significant increase in coming years.

Helminth infection has been demonstrated to induce a regulatory environment and control immunopathologies including inflammatory and allergic diseases, both in experimental models and in humans, but complications may develop, so identification of single immunomodulatory proteins is being used as a strategy to avoid potential side effects from a live parasite infection. However, only a few helminth-derived molecules have been reported. Calreticulin is a ubiquitous protein first described in the endoplasmic reticulum, involved in intracellular Ca++ homeostasis and in glycoprotein folding. Recently, several additional key functions have been reported in the cytoplasm, membrane, and as a secreted form. Our group has identified and cloned the Taenia solium calreticulin as a recombinant protein (rTsCRT) and showed that it induces a predominant Th2 response after oral administration. Therefore, we investigated the potential of rTsCRT to regulate disease severity and inflammation in an experimental murine model of colitis induced by dextran sodium sulfate (DSS).

Methods: Female 6–8-week-old Balb/c mice were randomly divided in 4 groups: groups 1 and 3 were orally treated with 40μg of purified rTsCRT in carbonate buffer daily for 7 days and groups 2 and 4 were given carbonate buffer alone. Groups 2 and 4 received in addition 3% DSS in the drinking water for 7 days. Clinical disease activity and weight was assessed daily and on day 7 mice were euthanized, colon length was measured, and spleen weight registered, as well as cytokine production by quantitative RT-PCR and protein production by flow cytometry. To assess the effect rTsCRT on chronic colonic inflammation, 4 cycles of DSS were intercalated with 4 cycles of water.

The recombinant protein was given weekly for 4 weeks prior to DSS administration and additional doses of rTsCRT were given 3 days after DSS during the water cycles.

After the last cycle of DSS, mice were euthanized and same variables as in the acute model were measured. Quantitative RT-PCR was assessed using a Roche LightCycler 2.0, commercially available Taqman probes were employed with a onestep protocol, and eukaryotic translation elongation factor 2 was used as housekeeping gene.

Results: Initially, DSS concentration and commercial brand were standardized. We observed inhibition of the taq polymerase and retro transcriptase in the samples obtained from DSS treated mice. Thus, a cleaning protocol by a double lithium chloride precipitation was standardized which prevented interference of DSS during retrotranscription and PCR amplification. From the two housekeeping genes analyzed, α actin and eukaryotic translation elongation factor 2, the latter showed a lower variation coefficient and more stable expression and was used as the housekeeping gene for normalization. Next, treatment with rTsCRT was analyzed. Mice that did not receive DSS had no signs of disease or inflammation, whereas DSS-treated mice showed high disease activity indexes consistent with colitis. rTsCRT treatment, significantly decreased the clinical activity index and the macroscopic inflammation, suggesting a clinical effect on both acute and chronic colitis. rTsCRT had an important effect on preventing weight loss in the chronic model. We are currently evaluating the cytokine expression and protein production to understand the immunological mechanisms involved in the anti-inflammatory response induced by rTsCRT. Preliminary results show that proinflammatory cytokines (IL-6, TNF-α, IL-1β) are diminished in rTsCRT-treated mice as compared to DSS alone.

Conclusions: Oral administration of rTsCRT improves the clinical disease activity index and the macroscopic markers on DSS-induced acute and chronic models of colitis. This is consistent with our previous reports on the TNBS prophylactic model, suggesting that rTsCRT could be used as a potential management treatment for IBD. The lower levels of proinflammatory cytokines that resulted from rTsCRT treatment could be achieved in other immune mediated pathologies, so further studies are needed to fully understand the immunological mechanisms involved.

This study was supported by a grant from PAPIIT IN223816.

Excreted/secreted products from a helminth parasite inhibits tumorigenesis on the early stages of colitis associated colorectal cancer

Blanca Estela Callejas Piña, Sandy Reyes Martínez, Ilse Chávez Soto, Cuauhtémoc Del Ángel Sánchez Barrera, Yadira Ledesma-Soto, Luis Ignacio Terrazas Valdés

Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico

aletse_bianca@hotmail.com; sandy_reyes25@comunidad.unam.mx; ilsebios01@gmail.com; angel.casb@hotmail.com; lesy_790413@yahoo.com.mx; literrazas@campus.iztacala.unam.mx

Keywords: Inflammation, stat3, Immunomodulation., Taenia crassiceps, Colitis associated colorectal cancer, Excreted secreted products

Colorectal cancer is the one of the most frequent causes of death by cancer and it is the third type of cancer worldwide. A subtype of colorectal cancer is that associated with exacerbate inflammatory response in colon. Clinic studies confirm that patients with ulcerative colitis (UC) have a major risk to develop colorectal cancer through a pathologic process of inflammation-dysplasia-cancer. The early stages of colorectal cancer are characterized by an increase in the production of inflammatory cytokines such as TNF-α, IL-6, IL-1β, IL17 and induction of NF-KB and STAT3 activation, mainly. These signaling pathways are necessary for proliferation and evasion of apoptosis by pre-neoplastic cells. Regulation of the inflammatory response in several diseases (colitis, arthritis, multiple sclerosis, etc.), through the use of helminth parasites or its derived molecules has been widely reported, however, the role that parasitic helminths play in the development of many neoplastic diseases associated with inflammation is still controversial. Previously, we reported that Taenia crassiceps (a helminth) infection reduces the development of colitis associated colorectal cancer (CAC). Nevertheless, the use of live parasites is still controversial in humans, thus many researchers have focused on the study of helminth-derived antigens as a novel source to treat inflammatory-mediated diseases. T. crassiceps excreted/secreted products (TcES) have been reported by down-modulate dendritic cell and macrophage activation in response to inflammatory stimuli. Pre-clinical assays using TcES treatment in diabetes type 1 and experimental multiple sclerosis demonstrated that TcES downregulted the signs and avoid the advance of such inflammatory diseases. Therefore, the aim of this study was to evaluate the role of TcES during the development of colorectal cancer that is an inflammatory-mediated disease. We used a well-established CAC model, through the initial administration of Axozymethane, followed by 3 cycles of Dextran Sulfate Sodium in drinking water (AOM/DSS). Female BALB/c mice were CAC-induced and

on the day 26 after AOM induction (after finishing the first DSS cycle) a group of mice started to receive i.p. TcES (dosis) three times a week until finalize CAC induction on day 68. At the end of CAC induction we observed that 100% of the CAC group (untreated mice) developed tumors, while only 50% of the group CAC+TcES develop small tumors. Moreover, a significant reduction in the number of tumors was observed in mice receiving TcES. Also the CAC+TcES group showed better conservation of colon morphology and maintained a greater number of of goblet cells compared to untreated CAC mice. During CAC induction we also evaluated, by flow cytometry, in circulation the percentage of inflammatory monocytes CD11b+Ly6G-Ly6ChiCCR2+ as well as their medium fluorescence intensity (MFI). After the DSS-cycle 2, we observed decrease in the expression of the CCR2 receptor in the CAC+TcES group and a lower percentage of these inflammatory cells in the DSS-cycle 3 compared to the CAC group. Spleen cells from both groups were cultured and stimulated with plate-bound anti-CD3 antibody for 48h, supernatants from these cells were evaluated for TNF- and IL-10 production. We found that TNF-a production was significantly reduced in the CAC+TcES group compared to the CAC group; while IL-10 production displayed a reverse pattern. On the other hand, we performed immunohistochemistry assays on colon tissues to detect the expression of B-catenin and Ki67, both markers associated with tumorigenesis. B-catenin and Ki67 were both significantly decreased in the CAC+TcES group compared to untreated CAC animals. Due to the importance of STAT3 signaling pathway deregulation in CAC development we decided to evaluate STAT3 phosphorylation in colon tissue during the development of this neoplastic disease. Our results obtained by western blot indicated that STAT3 phosphorylation in the CAC group increased gradually as tumorigenesis progressed, in contrast, mice receiving early treatment with TcES maintained STAT3 activation at normal levels respect to control (healthy mice). Similarly cyclin D1 expression was evaluated in colon tissue and it was found that CAC-induced mice displayed an increased expression of this cancer-marker compared to normal tissue. Remarkably, mice receiving TcES displayed a reduced expression of cyclin D1. Taken together our data suggest that administration of TcES during CAC development could work as a regulator of tumorigenesis in early stages of this pathology, where a reduction in the number of tumors and inflammatory monocytes was observed as well as a reduction of several cancer-related markers. Nevertheless, is necessary to identify the mechanisms of inhibition by TcES on STAT3 activation and others signaling pathways associated with inflammation and colorectal cancer development.

Evaluation of functional capacity to support lymphopoiesis by Mesenchymal Stromal/Stem Cells (MSCs) coming from neonatal tissues

Ixel Luna-Cañedo[1], Rosana Pelayo[2], Juan Carlos Balandrán[2], Víctor Adrián Cortés-Morales[1], Hector Mayani[1], Juan José Montesinos[1]

[1]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
ixel.luna@gmail.com; rosanapelayo@gmail.com; jcbalandran@gmail.com; v.adrian.cortes@gmail.com; hmayaniv@prodigy.net.mx; montesinosster@gmail.com

Keywords: Lymphopoiesis, Mesenchymal Stem Cells, NK cells, B cells, Mesenchymal stromal cell, Killer dendritic cells

Introduction: Mesenchymal Stromal/Stem Cells (MSCs) of neonatal tissues: placenta (PL), umbilical cord blood (UCB) and Wharton´s Jelly (WJ) have the capacity to support myelopoiesis. However, few studies have analyzed the rol of MSCs in lymphopoiesis. Thus, basic aspects of MSCs to regulate lymphopoiesis are poorly understood and for the near future, hematopoietic support capacity of MSCs from alternative sources to bone marrow (BM) are clinically important to improve hematopoietic stem cells transplants.

Objective: Evaluate the capacity of Mesenchymal Stem Cells from neonatal tissues to support lymphopoiesis.

Methods: MSCs were obtained from BM, UCB, PL and WJ samples and analized to determine their morphology, cell-surface antigen expression and osteogenic, chondrogenic and adipogenic differentiation potential. CD34+ CD38- cells obtained from UCB samples were isolated and co-culture on different subconfluent monolayers of MSCs from BM, UCB, PL and WJ. To determine B lymphocyte differentiation, co-culture of MSCs and CD34+ CD38- cells were incubated for 5 weeks in presence of a cytokine cocktail containing IL-7, SCF and FLT-3-L. Meanwhile co-cultures to determine NK cells and Dendritic Cell differentiation, were performed during 3 weeks in presence of SCF, FLT3-L, IL-15 and IL-7. After cultivation time, lymphoid populations were evaluate by flow cytometry to determine the expression of membrane markers. Co-cultures without cytokines were used as controls.

Results: MSCs were positive for CD105, CD73, CD90, and CD13 markers. MSCs from all sources showed osteogenic and chondrogenic differentiation, however those from UCB showed poor adipogenic differentiation potential compared with MSCs from BM, PL and WJ.

We observed in co-cultures that MSCs from all sources are capable to generate IKDCs, a subpopulation of NK cells recently characterized by their co-expression of CD56 and CD11c and their function as antigen-presenting cell like dendritic cells, cytotoxicity and anti-tumoral activity like NK cells. Interestingly, co-cultures with BM-MSCs were the only ones in which both populations of NK cells, cytotoxic (CD56 high) and regulatory (CD56 dim), were detected. In contrast, in co-cultures of MSCs from neonatal sources, only cytotoxic NK cells were generated. On the other hand, the highest generation of dendritic cells with phenotype CD11c+ CD11b+ was observed in co-cultures with UCB-MSCs. In a similar way, the highest detection of common dendritic cells whit CD16-CD11b+CD11c+ phenotype, was observed in co-cultures with WJ-MSCs. In the absence of cytokines, we only detected the generation of IKDCs, cytotoxic NK cells, CD11c+ CD11b+ and CD11b- CD11c+ dendritic cells in co-cultures with WJ-MSCs.

On the other hand, differentiation of B lymphocytes only was detected in co-cultures with PL-MSCs and UCB-MSCs. In both co-cultures, we observed the production of mature B lymphocytes with phenotype CD34-CD45+CD19+, but only in those with UCB-MSCs maintained also an immature B cell phenotype (CD34+ CD45+). No viable cells were observed in co-cultures in the absence of cytokines.

Conclusions: Our results indicate that MSCs from the studied sources have a differential capacity to generate lymphoid lineages, which determines distinctive biological characteristics of MSCs depending on the tissue from which they are obtained. Furthermore, such biological potential of MSCs could have implications in cellular therapy at the clinical level.

Targeting the human papillomavirus type 16 E5 oncoprotein to dendritic cells as therapeutic vaccine against cancer

Oscar Badillo-Godinez[1,2], Adolfo Pedroza-Saavedra[1], Lilia Chihu-Amparán[1], Fernando Esquivel-Guadarrama[2,3], Lourdes Gutiérrez-Xicoténcatl[1]

[1]Instituto Nacional de Salud Pública, Cuernavaca, Mexico
[2]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
[3]Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
badillo.oscar@gmail.com; apedroza@insp.mx; lchihu@insp.mx; fernando.esquivel@uaem.mx; mlxico@insp.mx

Keywords: Dendritic Cells, targeting, tumor, DEC-205, E5 HPV-16

The uterine cervical cancer is one of the most important public health problems worldwide. The human papillomavirus (HPV) is the main etiological agent for the development of cervical intraepithelial neoplasia (CIN) and cervical cancer (CC). The HPV codifies for three oncogenes E5, E6 and E7, which are specific tumor targets for vaccine design. However, there is a differential expression of these oncoproteins during the development of cancer. The E6 and E7 are expressed all along the different stages of the disease, while, E5 is expressed on premalignant lesions (CIN I-II) and condylomas. The E5 oncoprotein from HPV type 16 is a hydrophobic membrane protein of 83 amino acids, located at endoplasmic reticulum, Golgi, endosomal compartment and plasma membrane. One mechanism proposed for E5 transforming activity is that increases the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) in cell lines, and blocks the endosome acidification allowing the recycling of the receptors. On the other hand, HPV is a persistent virus that can remain in the host for a long time without symptoms, due to a weak immune response. The immune system is capable to eliminate HPV-infected cells, but about 10% of them evade the immune response. In this way, E5 interferes with the activation of T cells by down-regulating the MHC-I and II, which allows the establishment of a persistence infection, follows of the cellular transformation and finally the development of cancer. To study the immune response against HPV16-E5 a tumor mouse model was developed by transferring TC-1 cells, which express E5, into C57BL/6 mice. The model showed that one immunization with an E5 peptide, containing a cytotoxic T cell epitope, generated an immune response against the tumor cells and delays the tumor growth (Chen, et al., 2004).

Recently, a therapeutic model has been described, in which antigens are directed to dendritic cells (DCs), by conjugating the antigen to a monoclonal antibody (mAb)

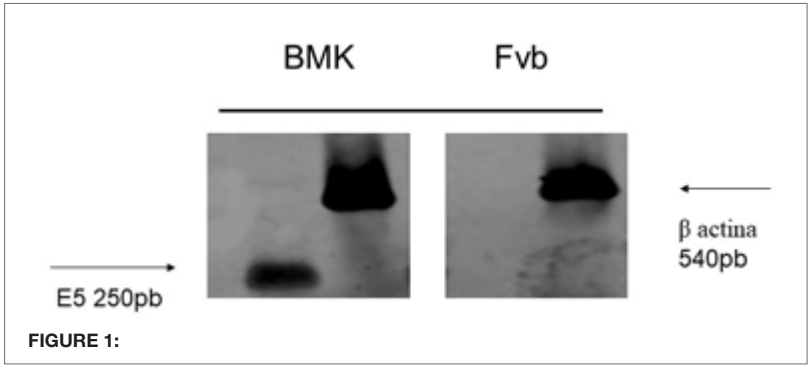


FIGURE 1:

specific for DEC-205, present on DCs. This system induces a potent T cell activation and a protective immune response at systemic and mucosal level (Bonifaz, et al., 2004; Badillo et al., 2015). The aim of the present work was to evaluate whether E5 targeted to the C-type lectin membrane protein DEC-205, present in the DCs, induce a protective immune response against tumor cells expressing the oncoprotein. Thus, a BALB/c HPV16 tumor mouse model was generated by transferred the transformed BMK16-myc cells that contain the complete HPV16 genome. The expression of E5 mRNA in the BMK16-myc cells was corroborated by RT-PCR, since a specific band of 250 bp was identified (Fig. 1). To generate the tumors, groups of 5 BALB/c mice were inoculate with 5x105 BMK16-myc cells s.c., and one week later were immunized as follows: A) Five μg of anti-DEC-205-E5 (chemically cross-linked) plus 50 μg of Poly I:C as adjuvant; B) Five μg of E5 alone plus adjuvant (control); and C) PBS plus adjuvant. Mice were immunized again one week later with the same treatment and follow up for up to 100 days by measuring weekly the tumor size. The results showed that mice immunized with DEC-205-E5, presented anti-tumor activity since these mice showed a reduction of tumor size between 10 to 15 times as compared to the E5 and PBS treated mice by 30 days (Fig. 2). In the DEC-205-E5 group the 33% of mice showed almost a complete clearance of the tumor and this was maintained up to 72 days after immunization. In contrast, the tumor-bearing mice immunized with E5 alone or PBS showed no reduction in tumor growth (tumor size around 500 mm3 and 600 mm3, respectively). These results showed that E5 targeted to DCs, through the DEC-205 receptor, could induce a strong protection against E5 and lead to a significant inhibition of tumor growth and increased survival (100 vs 30 days) in this murine tumor model. These results could contribute in the future for the design of a new generation of HPV therapeutic vaccines.

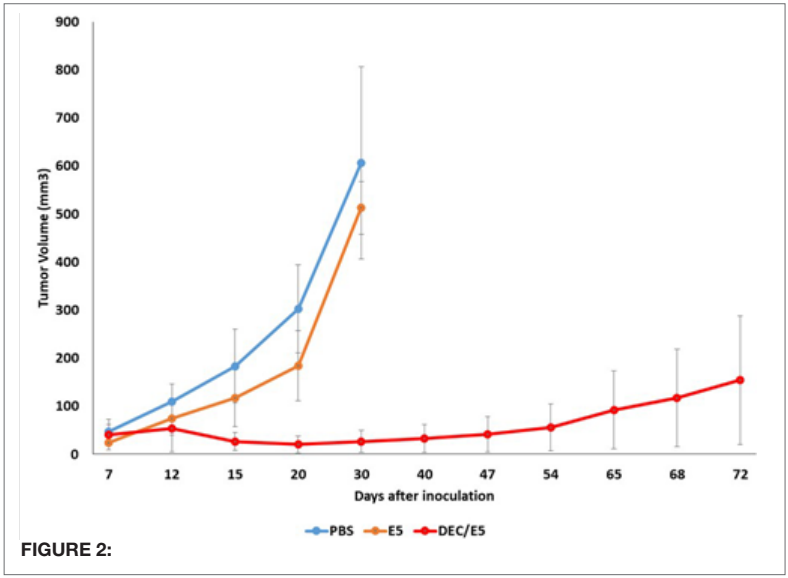


FIGURE 2:

REFERENCES

1. Chen YF, Lin CW, Tsao YP, et al. Cytotoxic-T-lymphocyte human papillomavirus type 16 E5 peptide with CpG-oligodeoxynucleotide can eliminate tumor growth in C57BL/6 mice. J Virol, 2004; 78(3): 1333-1343.
2. Bonifaz L C., B.D.P., Charalambous Anna, Darguste Dara I., Fujii Shin-Ichiro, Soares Helena, Brimnes Marie K., Moltedo Bruno, Moran Thomas M., and Steinman Ralph M. , In Vivo Targeting of Antigens to Maturing Dendritic Cells via the DEC-205 Receptor Improves T Cell Vaccination. The Journal of Exp Med, 2004; 199(6): 815-824.
3. Badillo-Godínez O., Gutierrez-Xicotencatl L., Plett-Torres T., Pedroza-Saavedra A, Gonzalez-Jaimes A., Chihu-Amparán L, Maldonado-Gama. M., Espino-Solís G., Bonifaz-Alfonzo L., Esquivel-Guadarrama F. Targeting of rotavirus VP6 to DEC-205 induces protection against the infection in mice. Vaccine. 2015; 33: 4228-4237

Multiepitope VLPs of B19 parvovirus reduce tumor growth and lung metastasis in aggressive breast cancer model

Angel De Jesus Jimenez[1], Leticia Moreno-Fierros[1], Ismael Bustos Jaimes[2]

[1]Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonóma de México, Tlalnepantla, Mexico

[2]Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

angel_jimenez@comunidad.unam.mx; lemofi@unam.mx; ismaelb@unam.mx

Keywords: breast cancer, mouse model, VLPs, B19 parvovirus, Multiepitopic recombinant protein, free cell assambly

In order to improve the response to treatment of Cancer patients, immunotherapy seeks to induce specific immune responses towards the tumor components; and thus stop or delay its growth. Several types of immunotherapy have been tested, such as cytokines, immune checkpoint inhibitors, adoptive cell transfer and numerous vaccination strategies. Despite its modest clinical effect, vaccines remain as an attractive approach due to their specificity, safety, treatment tolerance and their ability to induce durable clinical responses due to immune memory.

All types of vaccines require target antigens to which the response is directed, particularly for cancer vaccines, these antigens have been used in the form of peptides, nucleic acids, or antigen-bearing cells. However, for the induction of immune responses capable of controlling the disease it is necessary to induce strong and durable immune responses. Although the use of peptides and RNA has been shown to be capable of inducing strong immune responses, its half-life is short within the organism due to the presence of proteases and RNAses; in addition, its processing by non-immune cells carries the risk of induction of tolerance to antigens. For this reason, it is necessary to find strategies that allow to extend the half-life of the antigens and increase the uptake by professional antigen-presenting cells to ensure the induction of powerful and durable immune responses.

Several delivery systems have been used for this purpose, among them virus-like particles, which are multimeric compounds of viral capsid structural proteins have several advantages over other delivery systems: i) VLPs lack nucleic acids so they are not infective and possess desirable characteristics of biosecurity and biodegradation, ii) VLPs are able to induce both humoral and cellular immune responses, because

they are processed by cross-presentation in antigen presenting cells. Moreover, due to these characteristics the VLPs have been modified to carry tumor antigens and induce immune responses in cancer.

In present study we selected the B19 human parvovirus VLPs formed by VP2 protein, due to their production advantages and assembly characteristics. These VLPs can be produced in prokaryotic systems and are capable of assembling in a cell-free environment, they can be modified at their amino terminus to add sequences of approx. 250 a.a. without affecting their assembly in VLPs. In addition, once assembled these particles are highly thermostable and by their size of 20nm are able to enter and accumulate in lymphatic tissues where they can be processed by CD8+ dendritic cells to induce cytotoxic cellular responses.

For the design of the multiepitopic quimeric VP2 vaccine we chose epitopes of tumor-associated antigens: (survivin GWEPDDNPI) and neoepitopes of the mouse cell line 4T1 (Tmtc2 QGVTVLAVSAVYDIFVFHRLKMKQILP, Gprc5a FAICFSCLLAHALNLIKLVRGRKPLSW, Qars FPPDAINNF). These epitopes were coupled to the amino terminal region of the VP2 protein, this construct was expressed in strain E. coli BL21 (DE3) with good production yields (~ 70mg / L) and could be assembled into particles of ~ 20nm in diameter using only chimeric protein. These chimeric VLPs (50µg) were administered therapeutically on days 7, 14, 21 and 28 days after tumor implantation (3X103 cells 4T1) in 6–8 week old female BALB/c mice. Once the tumors were detected, the treatments were then applied intraperitoneally and peri-tumorally; and the tumor growth was monitored during 30 days with a digital caliper.

The animals were sacrificed at the end of this period and the formation of metastatic foci in the lung, cell populations in the spleen and tumor infiltrate were analyzed. The proliferation of lymphocytes from treated mice was analyzed by in vitro re-stimulation with cell lysates from the 4T1 cell line. Our results showed that the native VLPs were capable by themselves of delaying the tumor growth with respect to the group treated just with the vehicle. While the addition of the epitopes to these VLPs allowed a greater decrease in the tumor growth with respect to the mice treated with the vehicle or with the native VLPs. Moreover, all treated groups had a decrease in splenomegaly, that is presented in this model. Importantly, the analysis of metastatic foci in lung showed that only multiepitopic VLPs were able to reduce the number and size of metastasis. The mechanism to explain capacity of these VLPs to reduce metastasis rest to be determined. But we speculate it be explained due to their capacity to enter and accumulate in lymph nodes (due to their size 20 nm). Since the VLPs were injected peri-tumorally, we presume these VLPs were able to access the local lymph nodes and also were able to induce immune responses in the tumoral site. In accordance with this possibility, the local lymph nodes are also one of the first sites of invasion during the metastatic

process, so the immune responses in this site could eliminate the metastatic tumor cells. This result is of great importance due to the main role of metastases in cancer death.

When we analyzed the cell populations in the spleen, we observed that the treatment of multiepitopic VLPs re-established the percentages of CD4 and CD8 T lymphocytes similar to those found in healthy mice. In addition this treatment reduces the percentage of CD11b+ Gr1+ cells corresponding to the Myeloid-derived suppressor cells, both in the spleen and in the tumor infiltrate. These suppressor cells have an important role in tumor progression in situ and in the establishment of metastasis. When we analyzed the proliferation of splenocytes stimulated with lysate of the cell line 4T1, we observed that only the treatment with multiepitopic VLPs had a significantly higher proliferation than the native VLPs and the group of untreated mice, indicating that treatment with multiepitopic VLPs is able to induce specific cellular responses against tumor cells. These results show that parvovirus B19 VLPs are a viable option as a delivery system in cancer immunotherapy due to their low production costs, their ease of assembly and their ability to induce immune responses capable of delaying tumor growth and decreasing the establishment of metastasis.

Mesenchymal stem cells derived from human bone marrow, dental pulp, gingival tissue and periodontal ligament: In vitro evaluation of their differentiation and immunosuppressive capacities

MP DelaRosa-Ruiz[1,2], JJ. Montesinos-M.[1], E. Flores-Figueroa[1], G. Fragoso-Gonzalez[1], M. Castro-Manrreza[2], M. Alvarez.[3], Víctor Adrián Cortés Morales[1], Jessica Lakshmi Prieto-Chavez[1], Daniel Díaz Espinosa de los Monteros[2]

[1]21st Century National Medical Center, Mexico, Mexico

[2]National Autonomous University of Mexico., Mexico, Mexico

[3]Laboratorio de Biología Celular y Molecular, Facultad de Odontología, Universidad Nacional Autónoma de México, Mexico City, Mexico

pilardelaros@hotmail.com; montesinosster@gmail.com; kena.flores@gmail.com; gladis@biomedicas.unam.mx; elmar_ca@yahoo.com.mx; malvap6@gmail.com; v.adrian.cortes@gmail.com; lakshmi.litmus@hotmail.com; ddiaa@ciencias.unam.mx

Keywords: Immunotherapy, T cells, Tregs, MESE, immunorregulation, Mesenchymal st, mesenchymal periodontal ligament

Introduction: Mesenchymal stromal cells (MSCs) are multipotent cells, which can give rise to several cell types including osteoblasts, adipocytes, and chondroblasts. These cells can be found in a variety of adult tissues, such as bone marrow and oral tissues. In recent years, the biological properties of MSCs have attracted the attention of researchers worldwide due to their potential application for treating a clinical situation. Between these properties, MSCs are able to act on all cells of the immune system by the secretion of soluble factors and membrane molecules resulting in decreased proliferation and function of T-cells, therefore they are considered that exhibit important immunoregulatory potential.

Methodology: Isolation and culture of MSCs derived from bone marrow (BM-MSCs), dental pulp (DP-MSCs), gingival tissue (G-MSCs) and periodontal ligament (PDL-MSCs) samples, were obtained from 5 volunteer donors according to the ethical guidelines.

Characterization of MSCs

Immunophenotypic characterization and differentiation capacities of MSCs were performed with FITC, PE, or APC-conjugated monoclonal antibodies against CD73,

CD90, and CD45 (BD Biosciences, San Diego, CA) CD105, CD13, CD14 (Caltag, Buckingham, United Kingdom), HLA-ABC, HLA-DR, CD31, and CD34 (Invitrogen, Carlsbad, CA).

Adipogenic, chondrogenic and osteogenic differentiation was induced with a commercial induction medium. Adipogenic differentiation was determined by visualizing the presence of Oil Red O-stained (Sigma-Aldrich, St. Louis, MO) lipid vacuoles. Osteogenic differentiation was assessed by alkaline phosphatase staining. For chondrogenic differentiation analysis micromasses obtained after culture, were fixed, embedded and sliced. Cross sections were stained with Alcian blue dye (Sigma-Aldrich).

CD3+ T-cell collection

PBMC were obtained from the peripheral blood samples of four volunteer donors by density gradient with Ficoll-Paque Plus (specific gravity < 1.077g/mL; GE Healthcare Bio-Sciences AB, Uppsala, Sweden). CD3+ T cells were obtained by separation with human CD3 MicroBeads and MACS MS columns (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the supplier's instructions. The purity of the obtained CD3+ T-cell suspensions was determined by flow cytometry and only suspensions with purity greater than 97% were used in the experiments.

CD3+T cells were maintained in RPMI medium (RPMI 1640, 10% fetal calf serum, 2mM l-glutamine, 100 U/mL of penicillin, 100 mg/mL of streptomycin, and 100 mg/mL of gentamicin.

Stimulation of T cells

Cultured CD3+T cells were activated in RPMI medium with Dynabeads CD3/CD28 T-cell expander (Invitrogen) at a 1:1 ratio (25 mL of dynabeads/1x106 T cells). Lymphocyte proliferation was measured by 3H-thymidine uptake or by flow cytometry of carboxyfluorescein succinimidyl ester (CFSE)-labelled lymphocytes.

Cocultures of MSCs/CD3+ T cells

Cocultures of MSCs/ CD3+T cells were performed in the cell–cell contact in 24-well plates; CD3+T cells (0.5x105) were seeded and activated in the absence or presence of 0.5x105 MSCs from BM-MSCs, DP-MSCs, G-MSCs and PDL-MSCs (MSC:T-cell ratio 1:1). CD3+T cells that were activated in the absence of MSCs were used as positive controls for proliferation, cytokine secretion, and surface molecule expression.

Proliferation assays

After 3 or 4 days of T-cell activation, the (CFSE)-labelled lymphocytes proliferation was measured (CD3+, CD4+, and CD8+ T-cell). T cells that were activated in the absence of MSCs were used as positive controls and were set at 100% proliferation. The levels of proliferation observed in the cocultures were normalized to this control.

Results: Individual experiments from BM-MSCs, DP-MSCs, G-MSCs and PDL-MSCs (n= 5) displayed immunophenotypes and differentiation capacities similar to those reported previously. MSCs from the four sources expressed high levels of the characteristic MSCs surface markers CD105, CD90, and CD73 as established by the International Society for Cellular Therapy (ISCT). Further, the MSCs expressed low levels of HLA-ABC, were HLA-DR-negative, and did not express hematopoietic markers such as CD34, CD45, and CD14 or endothelial markers such as CD31. Meanwhile, MSCs from the four sources were capable of osteogenic and chondrogenic differentiation; however, only BM- and G- and PDL-MSCs showed adipogenic potential, unlike DP-MSCs, which had no such potential.

We isolated CD3+ T cells (purity ‡ 97%) that were activated with anti-CD3/CD28 in the presence or absence of DP-MSCs, G-MSCs and PDL-MSCs. Proliferation of activated T cells cultured in absence of MSCs was considered 100% of proliferation response (positive control). CD4+ and CD8+ T-cell proliferation is inhibited by cocultivation and cellular contact with DP-MSCs, G-MSCs and PDL-MSCs.

Expression of CTLA-4 in activated T cells cultured in absence of MSCs was considered as the positive control (Control). Basal expression of molecules in nonactivated T cells was considered as negative control. We observed that in cocultures with DP-MSCs, G-MSCs and PDL-MSCs significantly enhanced the percentage of CD4+CTLA-4+ cells.

The results showed that the presence of activated T cells increased the percentage of PD-L1+ MSCs (CD3-CD90+PD-L1+) in the cocultures, regardless of cell contact: DP-MSCs, G-MSCs and PDL-MSCs. GM-SCs and PDL-SC. All sources favored the generation of T-cell subsets displaying a regulatory phenotype CD4+CD25+CTLA-4+/FoxP3+.

Conclusions: MSCs from DP, G and PDL showed similar immunosuppressive potential properties compared with those from BM, this in terms of inhibition of T cell proliferation, expression of membrane markers and generation of T-cell subsets displaying a regulatory phenotype. Our results indicate that MSCs from dental tissues might be a potent and reliable candidate for future therapeutic applications. In addition, these cells are easy accessibility.

Arthritogenic peptides presented by tolerogenic dendritic cells are able to reprogram effector CD4+ T cell responses from rheumatoid arthritis patients

Elizabeth Camila Rivas Yañez[1], Jaxaira Maggi[1], Octavio Alexis Aravena[1], Katina Schinnerling[1], Lilian Soto[1,2], Oscar Neira[3], Diego Francisco Catalán[1], Juan C Aguillon[1]

[1]*Instituto de Ciencias Biomédicas, Universidad de Chile, Santiago, Chile*
[2]*Hospital Clínico de la Universidad de Chile, Santiago, Chile*
[3]*Salvador Hospital, Santiago, Chile*
elizabeth.ecry@gmail.com; jaxaira.maggi@gmail.com; aravena.octavio@gmail.com; katina.schinnerling@gmail.com; sotolian@gmail.com; oneira@med.uchile.cl; dfcatalan@med.uchile.cl; jaguillo@med.uchile.cl

Keywords: rheumatoid arthritis (RA), dendritic cells and regulatory T-cells, Self-antigens, tolerogenic dendritic cells (tDC), Regulatory T cells (Tregs)

Introduction: Rheumatoid arthritis (RA) is a highly invalidating autoimmune disease which generates inflammation in patient's joints leading to cartilage and bone destruction. Current treatments for RA have limited efficacy and are highly toxic due to their prolonged use, thus, new therapeutic approaches are necessary. Different cellular therapies have now developed to achieve disease remission, but these require permanent administration to maintain their effect. In the search for improving the clinical conditions of RA patients, regulatory T and B cells have been identified as paramount for control of the inflammatory process. These cells produce anti-inflammatory cytokines such as IL-10, IL-35 and TGF b, which mediate other lymphocyte activities and even block B cell responses against the same antigens, this way managing to lessen the immune response against a specific antigen relevant for RA. Dendritic cells (DCs) are professional antigen presenting cells capable of biasing immune responses from pro-inflammatory to tolerogenic in an antigen-specific manner, so these DCs with regulatory features or tolerogenic DCs (tolDCs) have become a potential therapy for RA. We have developed a protocol for tolDC generation, using dexamethasone as an immunomodulatory agent and monophosphoryl lipid A (MPLA) as a maturation agent, thus obtaining activated tDCs called MPLA-tDCs. MPLA-tDCs can suppress effector CD4+ T lymphocyte function and could allocate this modulation to other lymphocytes, reducing INFγ and IL-17 production. Due to RA-related antigens are not well known, the identification of specific arthritogenic antigens associated with RA development is of great relevance, since these are essential for tolerance inducing DC-based antigen-specific therapies development.

Aim: Our aim was to dilucidate the mechanism by which MPLA-tDCs challenged with self-antigens can induce the expansion of regulatory T cells capable of prompting responses of B cells specific for the same antigen.

Materials and Methods: Peripheral blood mononuclear cells (PBCM) from RA patients were stimulated with different peptide antigens to identify immunodominant arthritogenic antigens that are most recognized by immune cells of RA patients. A hemagglutinin (HA)-derived peptide was used as control. Additionally, mature DCs (mDCs) derived from monocytes from RA patients, were challenged with the antigen peptides and co-cultured with autologous CD4+ T cells for 6 days, after which their immune response was evaluated. The CD4+ T cell immune response from PBMC stimulation and mDCs/CD4+T cell co-cultures against these antigens were evaluated through CFSE dilution to measure cell proliferation, as well as by determination of CD25 and CD69 activation markers, and IFNgamma and TNFa intracellular expression. To evaluate if the immune response against these potential arthritogenic antigens could be modulated, MPLA-tDCs challenged with the antigens were co-cultured with total CD4+ T cells, memory CD4+ T cells or oligoclonal antigen-specific CD4+ T cells and their phenotype evaluated by flow cytometry. Moreover, MPLA-tDCs-modulated CD4+ T cells were then co-cultured with effector CD4+ T cells to see if the regulatory response could be further transferred to these cells.

Results: Out of 17 peptides selected from literature and prior studies by us, we identified 6 immunodominant peptides to which most samples of RA patients evaluated responded. 33% of RA samples responded against vimentin, fibrinogen, calreticulin and fibronectin. This was corroborated in co-cultures with mDCs, in which lymphocyte activation detected was found to be stronger than in PBMC samples. Antigen-specific cell lines against these antigens were successfully generated from RA patients` PBMC samples, exhibiting high activation markers and cytokine levels when stimulated with the specific antigen. This response seemed to be even higher when stimulation with the antigen was reiterated. On the other hand, antigen-specific CD4+ T cells co-cultured with autologous antigen-challenged MPLA-tDCs showed reduced proliferative and activation response, which was more notorious when antigen-specific cell lines were used. Furthermore, although no Foxp3 expression was detected, IL-10-producing Tr1 cells were found after incubation with MPLA-tDCs.

Conclusion: We identified 6 immunodominant arthritogenic antigens that induced a potent immune response in samples from RA patients. MPLA-tDCs loaded with these antigens were able to induce a regulatory phenotype in CD4+ T cells, further

supporting the use of these cells as a potential cellular therapy for tolerance restoration towards self-antigens in RA.

Support: Fondef ID15I10080 and Fondecyt 1181853.

Potential therapeutic application of newcastle disease virus for the treatment of lymphoma

Diana Sánchez[1], Rosana Pelayo[2], Marcela Lizano[1,3], Luis Alberto Medina[1], Eduardo Vadillo[4], Rogelio Sánchez-Verin[5], Gabriela Cesarman[6], Rosa Elena Sarmiento[1]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
[3]Ciencias Médicas F Subdirección de Investigación Básica - INCAN, Ciudad de México, Mexico
[4]Unidad de Investigación Médica en Enfermedades Oncológicas (UIMEO), Mexico, Mexico
[5]Hospital Español, Ciudad de México, Mexico
[6]Instituto Nacional de Cancerología (INCan), Tlalpan, Mexico
dianasanchezc@hotmail.com; rosanapelayo@gmail.com; lizanosoberon@gmail.com; medina@fisica.unam.mx; evadillo@hotmail.com; genryus_blade@hotmail.com; gcesarman@gmail.com; rosass@unam.mx

Keywords: Immunotherapy, Newcastle disease virus, Oncolytic Viruses, non-Hodgkin lymphoma, NDV, Canine lymphoma

Research on oncolytic viruses has mostly been directed towards the treatment of solid tumors, which has yielded limited information regarding their activity in hematological cancer. It has also been directed towards the treatment of humans, yet veterinary medicine may also benefit. Several strains of the Newcastle disease virus (NDV) have been used as oncolytics in vitro and in a number of in vivo experiments. We studied the cytolytic effect of NDV-MLS, a low virulence attenuated lentogenic strain, on a human large B-cell lymphoma cell line (SU-DHL-4), as well as on primary canine-derived B-cell lymphoma cells, and compared them to healthy peripheral blood mononuclear cells (PBMC) from both humans and dogs. NDV-MLS reduced cell survival in both human (42% ± 5%) and dog (34% ± 12%) lymphoma cells as compared to untreated controls. No significant effect on PBMC was seen. Cell death involved apoptosis as documented by flow-cytometry. NDV-MLS infections of malignant lymphoma tumors in vivo in dogs were confirmed by electron microscopy. Early (24 h) biodistribution of intravenous injection of 1 × 10^12 TCID50 in a dog with T-cell lymphoma showed viral localization only in the kidney, the salivary gland, the lung and the stomach by immunohistochemistry and/or endpoint PCR. We conclude that NDV-MLS may be a promising agent for the treatment of lymphomas. Future research is needed to elucidate the optimal therapeutic regimen and establish appropriate biosafety measures.

Arthritogenic peptides presented by dendritic cells re-establish tolerance

Elizabeth Camila Rivas Yañez, Jaxaira Maggi, Octavio Alexis Aravena, Paulina A. García-González, Katina Schinnerling, Lilian Soto, Diego Catalán, Juan Carlos Aguillón G.

Instituto de Ciencias Biomédicas, Universidad de Chile, Santiago, Chile
elizabeth.ecry@gmail.com; jaxaira.maggi@gmail.com; aravena.octavio@gmail.com; garcia.gonzalez.pa@gmail.com; katina.schinnerling@gmail.com; sotolian@gmail.com; diegocatalanm@gmail.com; jcaguillo@gmail.com

Keywords: rheumatoid arthritis (RA), dendritic cells and regulatory T-cells, Self-antigens, tolerogenic dendritic cells (tDC), Regulatory T cells (Tregs)

Introduction: Rheumatoid arthritis (RA) is a highly invalidating autoimmune disease which generates inflammation in patient's joints leading to cartilage and bone destruction. Current treatments for RA have limited effectivity and are high toxicity due to their prolonged use, thus, new therapeutic approaches are necessary. Different cellular therapies have now developed to achieve disease remission, but these require permanent administration to maintain their effect. In the search for improving the clinical conditions of RA patients, regulatory T and B cells have been identified as paramount for control of the inflammatory process. These cells produce anti-inflammatory cytokines such as IL-10, IL-35 and TGFb, which mediate other lymphocyte activities and even block B cell responses against the same antigens, this way managing to lessen the immune response against a specific antigen relevant for RA. Dendritic cells (DCs) are professional antigen presenting cells capable of biasing immune responses from pro-inflammatory to tolerogenic in an antigen-specific manner, so these DCs with regulatory features or tolerogenic DCs (tolDCs) have become a potential therapy for RA. We have developed a protocol for tolerogenic dendritic cell (tolDCs) generation, using dexamethasone as an immunomodulatory agent and monophosphoryl lipid A (MPLA) as a maturation agent, thus obtaining activated tolerogenic dendritic cells called MPLA-tDCs. MPLA-tDCs can suppress effector CD4+ T lymphocytes and could allocate this modulation to other lymphocytes, reducing INFγ and IL-17 production. To achieve this, DCs need to present a specific antigen towards which direct the immune response, and so far RA-related antigens are not well known. Therefore, identification of specific arthritogenic antigens associated with RA development is of great relevance, since these are essential for DC-based antigen-specific therapies by which we could inhibit the immune response.

Aim: Our aim was to dilucidate the mechanism by which MPLA-tDCs challenged with self-antigens can induce the expansion of regulatory T cells capable of prompting responses of B cells specific for the same antigen.

Materials and Methods: Mononuclear cells from peripheral blood (PBCM) of RA patients were stimulated with different peptide antigens (aggrecan, vimentin, fibrinogen, calreticulin and fibronectin) to identify immunodominant arthritogenic antigens that are most recognized by immune cells of RA patients. An hemagluttinin (HA)-derived peptide was used as control. Additionally, mature DCs derived from monocytes from RA patients, were challenged with these peptides and co-cultured with autologous CD4+ T cells for 6 days, after which their immune response was evaluated. The CD4+ T cell immune response from PBMC stimulation and mDCs autologous co-cultures against these antigens were evaluated through CFSE dilution to measure cell proliferation, as well as by determination of surface levels of activation markers CD25 and CD69 and intracellular cytokine levels of IFNgamma and TNFa, all by flow cytometry. In order to see if the immune response against these potential arthritogenic antigens could be modulated, MPLA-tDCs challenged with the antigens were co-cultured with total CD4+ T cells, memory CD4+ T cells or oligoclonal antigen-specific CD4+ T cells and their phenotype evaluated by flow cytometry as described before. Moreover, MPLA-tDCs-modulated CD4+ T cells were then co-cultured with effector CD4+ T cells to see if the regulatory response could be further transferred to these cells.

Results: Out of 17 peptides selected from literature and prior studies in our laboratory, we identified 6 immunodominant peptides to which most samples of RA patients evaluated responded. 33% of RA samples responded against vimentin, fibrinogen, calreticulin and fibronectin. This was corroborated in co-cultures with mDCs, in which lymphocyte activation detected was found to be stronger than in PBMC samples. Antigen-specific cell lines against these antigens were successfully generated from RA patients PBMC samples, exhibiting high activation markers and cytokine levels when stimulated with the specific antigen. This response seemed to be even higher when stimulation with the antigen was reiterated. On the other hand, antigen-specific CD4+ T cells co-cultured with antigen-challenged autologous MPLA-tDCs showed reduced proliferative and activation response, which was more notorious when antigen-specific cell lines were used. Furthermore, although no Foxp3 expression was detected, IL-10-producing Tr1 cells were found after incubation with MPLA-tDCs.

Conclusion: We identified 6 immunodominant artrhitogenic antigens that induced a potent immune response in samples from different RA patients. MPLA-tDCs loaded

with these antigens were able to induce a regulatory phenotype in CD4+ T cells in an antigen specific manner, further supporting the use of these cells as a potential cellular therapy for tolerance restoration towards self-antigens in RA.

Effects of Mesenchymal Stem Cells on endothelial dysfunction induced by Shiga toxin, the causative agent of Hemolytic Uremic Syndrome

Daiana Martire Greco[1,2], Carlos Luzzani[2], Luis A. Castillo[1], Verónica Alejandra Furmento[2], Alejandro La Greca[2], Nahuel Rodriguez Rodrigues[1], Veronica Landoni[1], Santiago Miriuka[2], Gabriela Cristina Fernandez[1]

[1]Instituto de Medicina Experimental, Academia Nacional de Medicina(CONICET), Ciudad autónoma de Buenos Aires, Argentina

[2]Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia, Buenos Aires, Argentina daianamartire@hotmail.com; carlosluzzani@gmail.com; luiscastillo718@gmail.com; alejandra_furmento@hotmail.com; ale.lagreca@gmail.com; nahuelrodriguezr@gmail.com; vilandoni@yahoo.com.ar; smiriuka@fleni.org.ar; gfernandez@hematologia.anm.edu.ar

Keywords: Mesenchymal Stem Cells, Shiga Toxin 2, VEGF, Hemolityc Uremic Syndrome, endothelial repairment

Introduction: Mesenchymal stem cells (MSC) are multipotent cells that possess known tissue regenerative properties. Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cell (iPS-MSC) has similar characteristics as MSC but can be obtained in larger quantities and more easily. Endothelial tissue damage in the renal glomerulus is one of the most relevant issues that cause kidney failure in Hemolytic Uremic Syndrome (HUS), wich is an endemic pathology in Argentina. Our aim was to investigate if iPS-MSC were able to contribute in the repair of endothelial damage cause by the exposure to lipopolysaccharide (LPS) and Shiga toxin 2 (Stx), the two most important etiological mediators in HUS.

Methods: We used an in vitro model of endothelial damage using the cell line HMEC-1 incubated with LPS and/or Stx for 24 h.

Results: We found that iPS-MSC exposed to LPS presented a decreased relative migrated area after a wound in the cell monolayer compared to Control cells, but the combination of LPS+Stx reversed this effect (relative area(x102), Control:5,10±0,04; LPS:3,60±0,02*; Stx: 3,80±0,03*;LPS+Stx:6,50±0,04#,*vs. Control and #vs. LPS or Stx, $p<0,05$). Also, LPS+Stx decreased the release of tumor necrosis factor-a (TNF-a measured by ELISA and increased the expression of the RNAm of Vascular Endothelial Growth Factor (VEGF) assessed by qPCR (pg/ml TNF-a Control:27±0,7; LPS:152±3,5*; Stx:75±2,7;

LPS+Stx: 79±0,7#; VEGF RNAm expressed in % to Control group, Control:1; LPS: 1,09±0,05*; Stx: 1,60±0,12;LPS+Stx:2,10±0,15#; *vs. Control and #vs. LPS p<0,05). Then, we studied the effect of iPS-MSC conditioned media (CM), obtained from the different groups, on endothelial cells. Only the CM from iPS-MSC treated with LPS+Stx increased the repair in a wound-healing assay (relative area, Control: 68±0,4; LPS:0,3±0,1*; Stx:0,2±0,03*;LPS+Stx:33±0,3 #,*vs. Control and #vs. LPS or Stx p<0,05). In conclusion, whereas LPS and Stx alone seems to be a pro-inflammatory stimuli for iPS-MSC, together LPS+Stx activate a repair endothelial program in iPS-MSC.

The unfolded protein response sensor IRE-1α modulates innate recognition and antigen presentation of melanoma tumor cells

Bernardita Medel, Cristóbal Costoya, Flavio Andres Salazar Onfray, Fabiola Osorio

Universidad de Chile, Santiago, Chile
bernardita.medel@ug.uchile.cl; ccostoya@ug.uchile.cl; flavio.salazar@inmunotron.med.uchile.cl; fabiolaosorio@med.uchile.cl

Keywords: cancer immunotherapy, cross-presenting DC's, IRE1α, unfolded protein response (UPR), Murine dendritic cells

Dendritic cells (DCs) are the most efficient antigen-presenting cells of the immune system. To fulfill this task, these cells regulate a variety of intracellular processes, including the unfolded protein response (UPR), which is a signal transduction mechanism involved in maintaining the fidelity of cellular proteome. The UPR sensor IRE-1α and XBP-1s, the transcription factor activated by IRE-1α-dependent splicing, are the most conserved among species. Besides the canonical role in UPR, IRE-1α/XBP-1 pathway also controls function and differentiation of immune cell types. We have previously shown that dendritic cells constitutively activate IRE-1α signaling and that this regulates the cross-presentation of dead cell-associated antigens. The latter aspect is highly applicable to cancer immunology, as most tumor antigens are likely to be contained within dying cancer cells. Thus, the aim of this work is to elucidate the contribution of IRE-1α signaling in the innate recognition and antigen-presentation of melanoma-associated antigens to T cells.

In this work, we show that human cancer melanoma lysates (MEL) elicit potent activation of the IRE-1α/XBP-1s pathway in BMDCs, along with additional members of the UPR including CHOP and BiP. This activation is mediated by a soluble factor contained in the lysates. To study the role of IRE-1α/XBP-1s pathway in DC function, we used 4μ8C, a specific inhibitor of IRE-1α RNAse domain. DCs stimulated with MEL in presence of 4u8C produced lower levels of pro-inflammatory cytokines and show curtailed abilities to cross-present antigens derived from MEL to CD8+ T cells. The latter effect is noticed in several types of DC in vitro cultures, suggesting that IRE-1α/XBP-1 pathway is a general regulator of cross-presentation. Specifically, IRE-1α appears to regulate the adjuvanticity and not the antigenicity of melanoma lysates as experiments decoupling antigen and adjuvant show that IRE-1α senses the stimulatory potential of MEL. Interestingly, this effect is specifically observed for cross-presentation

of melanoma antigens as IRE1 blockade does not interfere with the phagocytic capacity of the DCs, or the endogenous MHC-I presentation of antigens and the presentation via MHC-II. These data suggests that IRE-1α/XBP-1 pathway is necessary to elicit CD8+ T cell specific immunity against tumor antigens and can be a novel therapeutic target for tumor DC-based immunotherapies. Ongoing research is carried out to reproduce these results in an in vivo model to strengthen the use of IRE-1α/XBP-1 pathway like a novel target to develop immunotherapies.

Improvement of antitumor immune response by adding a HIF-1a inhibitor in the immunization against melanoma in mice

Ricardo Andrés León Letelier[1,2], Isaí Martínez Torres[3], Mario Politecnico Cancino[3], Constantino III López Macias[1], Laura Bonifaz Alfonzo[1]

[1]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[2]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[3]Instituto Politécnico Nacional, Mexico City, Mexico
cayoleon3@gmail.com; isai_mtz88@hotmail.com; mecancinod@gmail.com; constantino@sminmunologia.mx; labonifaz@yahoo.com

Keywords: Immunotherapy, Melanoma, adjuvant, cancer vaccine, HIF-1a

Background: Melanoma is the most aggressive skin cancer. Even though melanoma is immunogenic, it is clear that the natural immune response against melanoma is ineffective to eradicate it. Despite that, the manipulation of the immune system has been a promising therapeutic approach to eradicate the tumor. The manipulation to enhance the immune system to enable the induction of a more effective anti-tumoral response is known as an immunotherapy. There are many immunotherapy strategies, from immune check-points inhibitors to adoptive cell therapy of chimeric antigen receptors T cells.1 Although there are already eight immunotherapies approved by the FDA, clinicians are far from obtaining a proper treatment to melanoma. Different strategies are needed, and a promising approach is cancer vaccine.

Cancer vaccine has been one of the immunotherapy strategies used to improve the antitumor immune response. For the competent induction of an effective cancer vaccine, it is required the use of a proper adjuvant and a tumor-associated antigen.2 The adjuvant is crucial, since it activates antigen presenting cells (APC), allowing the priming of tumor-specific T cells. In this study we characterize the capacity of a novel adjuvant, THD03, to potentiate the anti-tumoral responses induced by an experimental cancer vaccine.

Although cancer vaccines enhance the immune response against tumor cells, Treg induction frequently down-regulate the effectors T cells.3 To induce a stronger antitumor immune response it is crucial to eliminate the induced Tregs. We propose a HIF-1a inhibitor (CAV04) to deplete Treg. CAV04 inhibits HIF-1a increasing its degradation. Since HIF-1 binds to FoxP3, a characteristic transcriptional factor of Treg, CAV04 could degrade FoxP3 indirectly, restraining the Tregs induced by the immunization. 4

Materials and Methodology: To evaluate our experimental adjuvant and the HIF-1a modulator in a melanoma model, mice were immunized with ovalbumin (OVA) and THD03, with or without CAV04, subcutaneously at the right flank, seven days before the melanoma challenge. After one week of the immunization, 2.5x105 cells of the melanoma cell line that expressed constitutively the antigen OVA (MO4) was injected at the right flank as well. Starting from day 5, the length and with width of the tumor was measure, to calculate its volume.

To determine if the immunization has therapy properties, mice were inoculated with MO4, and after ten days they were immunized intratumorally, with or without CAV04. A second immunization was performed five days after, as a boost.

To evaluate the HIF-1a expression with our CAV04, we add it into lymphocytes T CD4+ in vitro. Since our model is an immunization, we overexpressed HIF-1a in lymphocytes with agonist antibodies against CD3 and CD28. We activated lymphocytes T CD4+ with or without the transfection, and at day 5 and 7 we evaluate the median fluorescence intensity (MFI) with immunofluorescence.

Results: THD03 has the required adjuvant properties to induce an antitumoral response against melanoma in a mice model. The immunization with THD03 and OVA causes a lower tumor growth and an increase of the tumor-free mice than the control group PBS.

The immunization with CAV04 (OVA+THD03+CAV04) causes a lower tumor growth and an increase of tumor-free mice than the immunization alone (OVA+THD03) and the control groups (PBS, OVA, CAV04, OVA+CAV04).

The only group that had a therapy effect was OVA+THD03+CAV04; the tumor growth was lower than the other groups.

The CAV04 decreases the HIF-1a expression on activated lymphocytes T CD4+.

Discussion: Cancer vaccines required an adjuvant to activates APC and an antigen to skew the adaptive immune system. In this work we use OVA as an antigen and THD03 as an adjuvant. As a prophylactic measure, our immunizations employing OVA with THDO3 showed the capacity to diminish the tumor volume and the number of tumor burden mice compared to the control PBS group. Thus, THD03 has adjuvant properties in the anti-tumoral response in a melanoma model in mice. Interestingly, THD03 alone is also capable to reduce the tumor size, although is not as efficient as the combination with the antigen.

The combination of the immunization with CAV04 enhanced even more the anti-tumor immune response. The synergy found when the CAV04 is applied to the immunization, could be due to the lower number of Treg induced by the immunization. This will be evaluated in future experiments. However, this combination showed therapeutic effect. In the control group (PBS) and the immunotherapy group (THD03+OVA) the tumor growth was similar, whilst the combination therapy (THD03+OVA +CAV04) controlled the size of the tumor.

It is clear that the application of CAV04 truly helps the immunization. An immunization is capable to overexpress HIF-1a in T CD4+ cells. To mimic a T cell activation in an in vitro essay, we activated with anti-CD3 and anti-CD28 agonist antibodies. At the same time of the T cell activation we add CAV04 in the media. The activation clearly overexpressed HIF-1a in lymphocytes T CD4+. The activation and CAV04 combination diminish the expression of HIF-1a at day 7. We are working to further elucidate the mechanism of the CAV04 synergy in the melanoma model.

Conclusions: THD03 has adjuvant properties to induce an anti-tumoral response against melanoma in a mice model.

To add CAV04 on an immunization or in a cancer vaccine (THD03 + OVA) improves the immune response against melanoma in mice.

The CAV04 decreases the HIF-1a expression on activated lymphocytes T CD4+.

REFERENCES

1. G. Lizee, W. W. Overwijk, L. Radvanyi, J. Gao, P. Sharma, and P. Hwu, "Harnessing the Power of the Immune System to Target Cancer," vol. 64, no. 1, pp. 71–90, Jan. 2013.
2. A. Agha and A. A. Tarhini, "Adjuvant Therapy for Melanoma," Curr Oncol Rep, vol. 19, no. 5, pp. 1–9, Apr. 2017.
3. Perret R. et al. "Adjuvants That Improve the Ratio of Antigen-Specific Effector to Regulatory T Cells Enhance Tumor Immunity". Cancer Res. 2013;73(22);6597-6608.
4. Dang E.V. "Control of Th17/Treg Balance by Hipoxia-Inducible Factor 1". Cell. 2011;146;772-784.

Effect of human intravenous immunoglobulin on the expression of Fc gamma receptors in monocytes and granulocytes

Stephania Vázquez Rodríguez[1], Lourdes Andrea Arriaga Pizano[2], Arturo Cerbulo Vázquez[3], Armando Isibasi Araujo[2], Luis Antonio Jiménez-Zamudio[1], Roberto Arizmendi Villanueva[4]

[1]Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
[2]Centro Medico Nacional Siglo XXI, Mexico City, Mexico
[3]Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[4]Hospital de la Mujer, Mexico City, Mexico
svazrod@gmail.com; landapi@hotmail.com; cerbulo@hotmail.com; isibasi@prodigy.net.mx; lajimenezz@gmail.com; arineo_@hotmail.com

Keywords: Sepsis, Whole blood assay, FcγR, IvIg therapy, LPS stimulation

Human intravenous immunoglobulin (IVIg) is administered in low dose as antibody replacement therapy, and in high dose as immunomodulatory therapy for inflammatory diseases such as sepsis. Currently there have been many advances in the management of patients with sepsis, however, the mortality rate remains high (from 30% to 50% of cases). Due to its broad and potent activity against bacterial products, together with antibiotic therapy the use of IVIg has been proposed as an adjuvant treatment for sepsis, however, its adjuvant use for sepsis has been shown to be useful in adults, but not in neonates. Paradoxically, IVIg exercises pro and anti-inflammatory activities, depending on its concentration. The low-dose lead to pro-inflammatory activity while high doses lead to anti-inflammatory activity. Possible functional mechanisms of IVIg therapy includes the ability of polyclonal IgG to stimulate receptors through the crystallizable fraction of gamma immunoglobulins (FcγR) in myeloid cells, with the consequent positive or negative signal transduction that regulates phagocytosis, synthesis of inflammatory mediators, among others, however, these mechanisms are poorly explored in neonates.

To determine the effect of IVIg at low (5 mg / mL) and high (10 and 15 mg / mL) concentrations on the percentage, and level of expression of FcγR's in monocytes and granulocytes of neonate, their mother and unrelated adult.

Total umbilical cord blood (UCB, n=5) or peripheral blood of their mother (PPB, n=3) or unrelated adult (APB, n=5) were cultured only in the presence of LPS (1, 10 and 100 ng/mL), only IVIg (5, 10 and 15 mg/mL) or both (LPS + IVIg) in a kinetics of 0,

24 and 48 hours. Using flow cytometry, the level of expression of FcγR's in monocytes and granulocytes was determined. Monocyte and granulocyte immunophenotypes were determined using the Infinicyt analysis software (Cytognos Euroflow).

Whole blood (UCB, PPB or APB) with 1, 10 and 100 ng/mL of LPS, and/or with 5, 10 and 15 mg/mL of IVIg did not change the percentage of monocytes or granulocytes. It was observed that the expression of FcγRI and FcγRII increase in monocytes of APB treated with IVIg 10 mg/mL, while in UCB the expression of FcγRII decreases after 24 hours of treatment. In APB granulocytes, stimulated for 48 hours with IVIg 10mg/mL significantly increases the expression of FcγRIII since time 0. In contrast, the expression of FcγRII in granulocytes decreases after 24 hrs treated with IVIg at low or high concentration in APB, PPB and UCB since time 0. In response to the different concentrations of LPS the expression of FcγRIII increases at time 24 and 48 hours in maternal granulocytes of PPB since to time 0, while the expression of FcγRII in monocytes of PPB and UCB decrease to time 24 hours. We observed that the expression of FcγRIII increases in monocytes of PPB (pre-stimulated with LPS for 24 hours) and treated for 24 hours with IVIg 10 mg/mL.

Our data suggest that FcγR are differential affected by IVIg in adults, pregnant women and neonates. This effect is also differential in time. Both condition (IVIg concentration and time) must be take account when IVIg is use for immunodulation in the treatment of sepsis.

Cell-permeable Bak BH3 peptide expressed on the surface of Salmonella enterica induce chemosensitization of tumor cells

Armando Alfredo Mateos-Chavez, Juan Daniel Castro Duarte, Omar Ugarte-Alvarez, Paola Muñoz-Lopez, Daniel Dimitri Hernandez-Cueto, Carlos Alberto Aguilar-Gonzalez, Rosendo Luria-Perez

Hospital Infantil de México Federico Gómez, Mexico City, Mexico
armandochavez.m@ciencias.unam.mx; danielduarte2293@hotmail.com; orugarte@hotmail.com; paolamlopez10@gmail.com; danteligueri@gmail.com; carlosuami@gmail.com; rluria@protonmail.com

Keywords: Salmonella enterica, Cancer, cancer immunotherapy, Live-attenuated bacterial vectors, Bak BH3 Peptide

Background: Cancer constitutes a public health problem worldwide and, even though patient's survival has substantially improved with usual treatments, drug's resistance favor failure of these treatments, and make necessary the search for new and most effective antitumor therapeutic alternatives for completely eradicate transformed cells. Cancer cells resistant to chemotherapy are characterized by the overexpression of antiapoptotic members of the Bcl-2 protein family such as Bcl-XL, Bcl-2 and Mcl-1. We recently demonstrated that cell-permeable peptides derived from BH3 domain of Bax protein could be used to block the antiapoptotic activity of Bcl-2 protein family, and induce chemosensitization in tumors cells. However, the peptides derived from BH3 domain of Bak protein required to be analyzed. The use of peptides in antitumor therapy has some inconveniences such as peptide stability after administration, and their effective and selective direction toward tumor cells. To overcome these problems, in this work we use Salmonella enterica, a live-attenuated bacterial vector, that can migrate into the tumor microenvironment, this bacterial vector will serve as delivery system for permeable peptides from BH3 domain of Bak protein.

Objective: Evaluation of the cell-permeable Bak BH3 peptide expressed on the surface of Salmonella enterica to induce chemosensitization in tumor cells.

Material and Methods: Using Recombinant DNA Technology, we obtained the recombinant Salmonellas that carry o release the Bak BH3 peptide on its surface. The Bak BH3 peptide was genetically conjugated to a Fusogenic peptide in order to make it cell-permeable. The recombinant protein production was assessed by SDS-PAGE, Western blot,

Immunofluorescence and Flow cytometry. Viability assays of tumor cells lines (from Non-Hodgkin Lymphoma and Acute Lymphoblastic Leukemia) infected with different recombinant Salmonellas (MOI=100), in presense or absence of Cisplatinum as chemotherapeutic agent, was analyzed by Tripan blue staining. Induction of Apoptosis in tumor cells infected with different recombinants Salmonellas (MOI=100), in presense or absence of Cisplatinum as chemotherapeutic agent was evaluated by active caspase-3. Combinations of recombinant Salmonella that carry and release the Bak BH3 peptide in presense or absence of Cisplatinum and the synthetic cell-permeable Bax BH3 peptide were also evaluated.

Results: The recombinant Salmonella strains that carry and release the cell-permeable Bak BH3 peptide were obtained. The expression of the recombinant proteins revealed the expected approximately 70 KDa in all constructs. All recombinant proteins were translocated to the Salmonella enterica surface through the MisL autotransporter system. A reduction in the tumor cells viability was detected when cells were infected with Salmonella enterica that carry and release the cell-permeable Bak BH3 peptide in presense of cisplatinum, while the apoptotic rates increased. The treatment using combinations of recombinant Salmonella that carry and release the Bak BH3 peptide in presense of Cisplatinum and the synthetic cell-permeable Bax BH3 peptide showed an increase in cell death.

Conclusions: Assays in the cell viability and apoptosis of tumors cells lines suggest that the use of Salmonella enterica as delivery system, of the cell-permeable Bak BH3 peptide that block the antiapoptotic activity of Bcl-2 protein family, is efficient for inducing apoptosis and chemosensitization of tumor cells lines, and this effect is enhanced in the presence of the synthetic cell-permeable Bax BH3 peptide.

Monoclonal antibodies specifically targeting protein β-sheet secondary structure can diminish toxic oligomers and pathology in neurodegenerative diseases

Fernando Goni, Mitchell Marta-Ariza, Krystal Herline, Frances Prelli, Henrieta Scholtzova, Thomas Wisniewski

New York University, New York City, United States
fernando.goni@nyumc.org; Mitchell.MartaAriza@nyumc.org; krystal.herline@nyumc.org; frances.prelli@nyumc.org; henrieta.scholtzova@nyumc.org; thomas.wisniewski@nyumc.org

Keywords: Cognitive Therapy, neurodegenerative disease, Animal Models, monoclonal antibodies, oligomeric neuroconformers

The pathogenesis of many neurodegenerative diseases (NDD) involves misfolding of a protein or peptide from a soluble physiological conformer to a pathological oligomeric form that can spread in a prion-like manner before contributing to insoluble fibrillar deposits. Synaptic and cellular toxicity is mainly associated with the misfolded oligomeric conformers. The most common NDD, Alzheimer's disease (AD), is characterized by two different misfolded proteins: the extracellular Aβ and the intracellular hyperphosphorylated tau (ptau) in paired helical filaments (PHF). While the Aβ deposits form neuritic plaques and congophilic amyloid angiopathy (CAA), the ptau-PHF forms neurofibrillary tangles (NFTs). Other NDD include Parkinson's disease (PD), Lewy body dementia (LBD) and prion diseases where the pathogenesis is associated with a similar aggregation/oligomerization process of α-synuclein and PrPRes respectively. Currently, there is no effective disease modifying pharmacological or immunological therapeutic approach for most NDD, with most clinical trials for AD producing disappointing results.

We have developed novel mouse anti-β-sheet conformational monoclonal antibodies (aβComAb) that specifically recognize only the dominant secondary structure on pathological oligomers of neurodegenerative diseases independently of the primary structure of the different conformers. Unusually, they are all stable IgMk pentameric (IgMkp) that histochemically distinguish intra- and extracellular pathology in human AD brains, LBD-PD brains, and the genetic human prion disease Gerstmann-Straussler-Scheinker (GSS), but failed to show any reactivity on young human brains with no related pathology. Two aβComAbs showed on surface plasmon resonance

(SPR) specific binding only to oligomeric Aβ with a KD in the nanomolar range. In ELISA assays and blots they recognized different oligomeric forms of human Aβ, PHF, α-synuclein and PrPres. An intact IgMkp aβComAb inoculated intraperitoneally (i.p.) in mice was able to cross the blood brain barrier (BBB), peaking at 24 hours post inoculation in the soluble brain fraction (~1% of the inoculated antibody) with 70% of the intact immunoglobulin still remaining in the soluble brain after 48 hrs. Aged triple transgenic (APP/PS1-tauP301L) AD mice (3xTgAD) with corroborated preexisting behavioral deficits and extensive Aβ and tau pathology, were weekly infused i.p. for two months with an aβComAb. Treated mice exhibited significant cognitive rescue on radial arm maze testing, compared to pre-inoculated animals and vehicle control infused mice. Immunohistochemically, treatment resulted in a significant decrease of Aβ and tau extracellular pathology without reductions in intracellular tau pathology. Biochemically, the treatment resulted in significant reductions of different oligomeric forms of Aβ and tau, including high molecular weight oligomers, not previously detected. No side effects were seen in the treated animals during the infusion time and at post-mortem examination. There were no microhemorrhages or indications of increased inflammation, associated with treatment. These results demonstrate that an intact pentameric IgM can cross the BBB, and exert a therapeutic action inside the brain, in the absence of toxicity. The specific aβComAbs may be suitable candidates to be humanized for clinical trials and be disease modifying therapeutic agents for Alzheimer's Disease or other related NDD.

Cacalolides from the medicinal plant species Psacalium decompositum and P. peltatum inhibit FcεRI-dependent degranulation in mast cells by the blockage of ROS

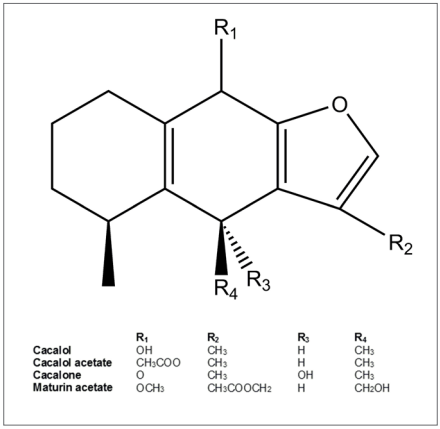
Jorge Ivan Castillo-Arellano[1], Silvia Laura Guzmán-Gutiérrez[2], Alfredo Ibarra-Sánchez[1], Ricardo Reyes-Chilpa[3], Claudia González-Espinosa[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico
[2]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[3]Instituto de Química, Universidad Nacional Autónoma de México, Coyoacan, Mexico
jorge.ivan@ciencias.unam.mx; laurettaguzz@yahoo.com.mx; alfreedoibarra@gmail.com; chilpa@unam.mx; cgonzal@cinvestav.mx

Keywords: Reactive Oxygen Species, Mast cell degranulation, Cacalol, Cacalone, Psacalium decompositum, cacalol acetate, maturin acetate, calcium channel regulation, Psacalium peltatum

Mast cells are important effectors in allergic reactions since they produce a number of pre-formed and de novo synthesized inflammatory compounds in response to high affinity IgE receptor (FcεRI) crosslinking with antigen leading to production of reactive oxygen species (ROS) and degranulation of vesicular content, releasing proinflammatory molecules: histamine, β-hexosaminidase, serotonin and interleukins as TNFα, which favors inflammation and recruitment of other leukocytes. IgE/Antigen-dependent degranulation and cytokine synthesis of this cell type have been recognized as relevant pharmacological targets for the control of deleterious inflammatory reactions. Despite the relevance of allergic diseases worldwide, pharmacological control of mast cell degranulation has not been achieved.

Cacalolides are sesquiterpenes produced in the rhizome of various plant species of the genera Psacalium, which are used by Raramauri ethnia of Mexico for treating rheumatism, but are also a remedy for common cold, diabetes and kidney ailments. The most studied compound is the cacalol has been identified as the major active compound in these species with antioxidant, antimicrobial and anti-inflammatory properties in vitro and in vivo. Cacalol is quickly oxidized to cacalone, which have been shown also to have anti-inflammatory activity. However, little has been studied regarding its anti-allergic activity, like other similar compounds that have recently been identified in P. peltatum such as maturin acetate.



	R_1	R_2	R_3	R_4
Cacalol	OH	CH_3	H	CH_3
Cacalol acetate	CH_3COO	CH_3	H	CH_3
Cacalone	O	CH_3	OH	CH_3
Maturin acetate	OCH_3	CH_3COOCH_2	H	CH_2OH

In this work we present the first in vitro approach of anti-allergy activity of four cacalolides: cacalol, cacalol acetate, cacalone and maturin acetate on the main activation parameters of this cell type utilizing bone marrow-derived mast cells (BMMCs) and generate the molecular mechanism of inhibition.

Among the four compounds evaluated, cacalol showed the highest inhibitory activity on IgE/Antigen-dependent degranulation with 88% (30 μM), followed by maturine acetate and cacalol acetate with an inhibition of 100% and 85% (100 μM) and finally cacalone with a 100% inhibition (300 μM). Additionally, cacalol between 30-300 μM concentrations was capable to almost completely inhibit the production of ROS in mast cell challenged with antigen dinitrophenyl hapten conjugated to Human Serum Albumin (DNP-HSA), as compared with the reference antioxidant Trolox (5 mM). Finally, cacalol showed that it is capable of inhibiting about 40% of the intracellular calcium [Ca2+]i mobilization required for IgE/antigen-induced degranulation between 0.3 and 3 μM, while completely inhibited [Ca2+]i mobilization at 30 μM.

The obtained results suggest that cacalol has a higher activity than other similar sesquiterpene compounds, probably due to its unstable chemical structure, which may allow to quickly decompose towards other more stable compounds while assimilating electrons derived from ROS produced by NADPH oxidase in mast cells, which are important for the activation of membrane calcium channels dependent on the Receptor Operated Calcium Entry (ROCE) and Stored Operated Calcium Entry (SOCE).

Attenuated Salmonella as neoadjuvant therapy for melanoma-bearing mice undergoing chemotherapy

María Moreno, Sofía Chilibroste, Amy Elizabeth Mónaco, María Magdalena Vola, Caroline Agorio, Jose Alejandro Chabalgoity

Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
mmoreno@higiene.edu.uy; sofiachilibroste@gmail.com; amonaco@higiene.edu.uy; magdalenavola@gmail.com; agorionor@gmail.com; jachabal@higiene.edu.uy

Keywords: Dacarbazine, Melanoma, Neoadjuvant Therapy, Salmonella, mouse model

Melanoma is a severe form of skin cancer with the highest incidence rate increase in the world. To date no treatment has been proven to be effective in the later stages of the disease. In the last few years, two new therapies were approved for the treatment of metastatic melanoma: antibodies against immune checkpoints, such as CTLA-4 and PD-1, and molecular inhibitors of BRAF mutations. However, its uses are limited because major side effects, highly elevated costs and induction of resistance to treatment. Even though, immunotherapies are still considered as the most convenient approach for the treatment of cancer since they can elicit long lasting immune response. Furthermore, combination between immunotherapies or with another sort of therapy such as chemotherapy or radiotherapy helps to restrain tumor plasticity to acquire resistance.

We have previously demonstrated that live attenuated Salmonella-based immunotherapy could be considered as a potential treatment for melanoma and B cell non-Hodgkin lymphoma (B-NHL). Intratumoral administration of Salmonella enterica serovar Typhimurim, LVR01 (aroC-), retards tumor growth and dissemination, and thereby prolongs animal free of disease and overall survival. LVR01 elicits systemic humoral and cellular immune response against tumor cells (Grille et al., 2014; Vola et al., submitted). Moreover, the administration of LVR01 to B-NHL-bearing mice under CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone treatment) retards tumor growth and prolongs free of disease and overall survival. Interestingly, Salmonella improves overall health status of animals undergoing chemotherapy (Bascuas et al., 2018).

In this work, we evaluate the potential of Salmonella LVR01 as neoadjuvant therapy in combination with dacarbazine, first line standard treatment of metastatic melanoma, in a murine melanoma model.

C57BL/6 mice were subcutaneously inoculated with B16F1 melanoma cells. Animals were divided into 4 groups: control, Salmonella (LVR01), dacarbazine (DTIC) and combined therapy (LVR01 plus DTIC). When tumors were palpable (day 10 post-tumor inoculation, pti), S. Typhimurium LVR01 (1x106 CFU) was intratumorally injected. At the following day (11 pti), chemotherapy treatment consisting in daily intraperitoneally application of 150mg/kg/doses dacarbazine was started and continued for 3 days.

The combined treatment retarded tumor growth and prolonged overall survival compared to control group ($p<0.0001$, log-rank test), and to both Salmonella- and dacarbazine-monotherapy groups ($p=0.0005$ and $p=0.0008$, log-rank test, respectively). The median survivals were 24, 32, 34 and 42 days for control, LVR01, DTIC, and LVR01 plus DTIC groups, respectively. It is worth to mention that 2 out of 12 combined therapy-treated animals completely recovered.

The combined therapy was well tolerated, with less that 10% of lost weight. Dacarbazine treatment induced a marked reduction in spleen and tumor draining lymph node (TDLN) sizes, while Salmonella induced splenomegaly and TDLN swelling. Spleens and TDLNs from combined therapy-treated animals recovered their normal appearance.

Regarding the immune response elicited by the combined therapy, increased expressions of ccl2, ccl5, cxcl9 and cxcl10 mRNA levels were found at day 27 pti. Concordantly, an increase in tumor infiltrating NK cells and neutrophils was found. Furthermore, tumor infiltrating cytotoxic lymphocytes, such as NK, NKT and CD8 T cells, expressed higher levels of CD69 and NKG2D, mainly due to dacarbazine treatment. A higher percentage of NKT and CD8 T cells was found in TDLNs of animals receiving LVR01 plus DTIC. Particularly, effector CD8 T cell population was expanded by the treatment. In addition, splenic NK cells from the treated animals exhibited higher cytotoxic activity against NK-sensitive YAC1 cells.

In conclusion, Salmonella immunotherapy could be safely used in individuals under chemotherapy treatment. The combined therapy induced activation of both innate and adaptive cytotoxic lymphocytes, resulting in longer survival. We believe that the use attenuated Salmonella as a non-specific active immunotherapy combined with standard chemotherapy in melanoma would be an interesting alternative therapeutic strategy, which could be easily moved into clinical trials.

REFERENCES

Grille S, Moreno M, Bascuas T, Marqués JM, Muñoz N, Lens D, Chabalgoity JA. Salmonella enterica serovar Typhimurium immunotherapy for B-cell lymphoma induces broad anti-tumour immunity with therapeutic effect. Immunology 143(3), 428–437 (2014).

Vola M, Mónaco AE, Bascuas T, Rimsky G, Agorio C, Chabalgoity JA, Moreno M. TLR7 agonist in combination with Salmonella as an effective anti-melanoma immunotherapy. Submitted.

Bascuas T, Moreno M, Grille S, Chabalgoity JA. Salmonella immunotherapy improves the outcome of CHOP chemotherapy in non-hodgkin lymphoma-bearing mice. Front Immunol., in press (2018)

Specific active immunotherapy with a VEGF targeted vaccine: from bench to bedside

Yanelys Morera

Center for Genetic Engineering and Biotechnology, Havana, Cuba
yanelys.morera@cigb.edu.cu

Keywords: Cancer, VEGF, Angiogenesis, Clinical Trial, Therapeutic vaccine, active immunotherapy

HeberSavax is a cancer therapeutic vaccine, based on recombinant modified human vascular endothelial growth factor (VEGF) as antigen, in combination with the adjuvants VSSP or alum. HeberSavax has shown to inhibit tumor growth and metastases in mice, and to induce VEGF blocking antibodies and specific T cell responses in several animal species, all with an excellent safety profile (1-5).

After preclinical studies, two phase I clinical trials were done where safety, tolerance, and immunogenicity of HeberSavax were studied in patients with advanced solid tumors(6-8). HeberSavax was found to be safe and tolerable in the final trial evaluation. Immunized patients had serum specific anti-VEGF IgG antibodies, the ability to block VEGF-VEGF Receptor 2 (KDR) interaction in an in vitro competitive ELISA assay, and positive IFN-gamma ELISPOT tests after in vitro peripheral blood mononuclear cell (PBMC) stimulation with a mutated VEGF molecule. The highest antigen dose vaccine combination produced the best results in the three specific immune response assays done, with higher frequencies of positive patients and individual test values.

Patients surviving the trial were eligible to start off-trial voluntary supervised HeberSavax re-immunizations every 4 weeks with the best antigen dose. We presented evidences of clinical benefit, and positive results in the specific immune response tests, for patients that had been off-trial re-immunized for close to six years. Our results indicated that after sustained vaccination most of the patients conserved their seroconversion status. The specific anti-VEGF IgG titer diminished, but in all the cases keeps values up from the pre-vaccination levels. Continued vaccination was also important to produce a gradual shift in the anti-VEGF IgG response from IgG1 to IgG4 (8). Outstanding, our results indicated that long-term off-trial vaccination could be associated with the maintaining of one reserve of antibodies able to interfere with the VEGF/Receptor interaction and the production of IFN gamma secretion in CD8+ cells.

The results derived from the study of this series of patients suggest that long term therapeutic vaccination is a feasible strategy, and highlight the importance of continuing

the clinical development program of this novel cancer therapeutic vaccine candidate. We also highlight the future clinical applications of HeberSavax in cancer and explain knowledge gaps that future studies may address.

REFERENCES

1. Morera Y, Bequet M, Ayala M, Lamdán H, Agger EM, Andersen P, et al. Antitumoral effect of active immunotherapy in C57BL/6 mice using a recombinant human VEGF protein as antigen and three chemically unrelated adjuvants. Angiogenesis 2008;11:381–93.
2. Bequet M, Morera Y, Ayala M, Ancízar J, Soria Y, Blanco A, et al. CIGB-247: a VEGF-based therapeutic vaccine that reduces experimental and spontaneous lung metastasis of C57Bl/6 and BALB/c mouse tumors. Vaccine 2012;30:1790–9.
3. Pérez Sánchez L, Morera Diaz Y, Bequet-Romero M, Ramses Hernández G, Rodríguez Y, Castro Velazco J, et al. Experimental studies of a vaccine formulation of recombinant human VEGF antigen with aluminum phosphate. Human Vacc Immunother 2015;11:2030–7.
4. Morera Y, Bequet M, Ayala M, Velazco JC, Pérez PP, Alba JS, et al. Immunogenicity and some safety features of a VEGF-based cancer therapeutic vaccine in rats, rabbits and non-human primates. Vaccine 2010;28:3453–61.
5. Morera Y, Bequet M, Ayala M, Pérez PP, Castro J, Sánchez J, et al. Antigen dose escalation study of a VEGF-based therapeutic cancer vaccine in non human primates. Vaccine 2012;30:368–77.
6. Gavilondo JV, Hernández F, Ayala M, de la Torre AV, de la Torre J, Morera Y, et al. Specific active immunotherapy with a VEGF vaccine in patients with advanced solid tumors. Results of the CENTAURO antigen dose escalation phase I clinical trial. Vaccine 2014;32:2241–50.
7. Morera y, Sánchez J, Bequet-Romero M, Selman-Housein, KH, de la Torre, A et al. Specific humoral and cellular immune responses in cancer patients undergoing chronic immunization with a VEGF-based therapeutic vaccine. Vaccine 35 (2017) 3582–3590.
8. Sánchez J, Morera Y, Bequet-Romero M, Hernández-Bernal F, Selman-Housein KH, de la Torre H et al., Characteristics of the specific humoral response in patients with advanced solid tumors after active immunotherapy with a VEGF vaccine, at different antigen doses and using two distinct adjuvants. BMC Immunology (2017) 18:39 DOI 10.1186/s12865-017-0222-z.

Adenoviral vectors coding pro-inflammatory cytokines as therapy for tuberculosis

Sujhey Hernandez Bazan, Octavio Ramos-Espinosa, Dulce Adriana Mata, Brenda Marquina-Castillo, Jorge Alberto Barrios-Payan, Rogelio Hernandez Pando

Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
sujheyhb@yahoo.com.mx; octavioramos13@gmail.com; dulmat@yahoo.com.mx; brenda.marquina@gmail.com; qcjbp77@yahoo.com.mx; rhdezpando@hotmail.com

Keywords: Gene Therapy, Immunotherapy, Osteopontin, Tuberculosis, mouse models, TNF-α, Adenoviral vector

Mycobacterium tuberculosis (Mtb), the main causal agent of tuberculosis (TB), can produce progressive disease or latent infection. In high endemicity countries, the first mycobacterial infection occurs in childhood and in most cases is controlled by the immune system. Only 10% of these primary infection cases lead to progressive disease. However, the treatment is long and requires the use of highly toxic drugs, specially for multi-drug resistant bacteria (MDR), so it is frequently abandoned favoring the arise of resistant bacteria. Thus, it is important to seek for new therapies, like gene therapy based in adenoviral vectors which is a low-cost and effective way to deliver any type of molecule codified in a gene. In this work, adenoviral vectors that codified for pro-inflammatory molecules were tested as a therapy to regulate the immune response to favor the elimination of micobacteria.

BALB/c mice were infected with 2.5x105 of Mtb bacteria strain H37Rv and 3.4x105 MDR clinical isolate, in independent experiments. After 60 days post-infection, adenoviral vectors codifying for mouse TNF-alpha (AdTNF) and osteopontin (AdOPN) were administered in separate groups at only one dose of 5x107PFU, also a group was administered with an empty vector as a control (AdGFP). After 28 and 60 days post-treatment, mice were euthanized and lungs were analyzed for bacteria load and tissue damage, resulting in a decrease of bacillary burdens at day 28 after treatment with AdTNF and AdOPN in mice infected with the MDR bacteria. For AdOPN this diminish bacterial load was sustain until day 60 after treatment not being the same for AdTNF, also lung damage was reduced with both treatments being a lesser percentage of pneumonia in mice treated with AdOPN. Mice infected with the H37Rv strain had lesser bacterial load in both treatment groups rather this differences are not statistically significant.

In conclusion, the administration of proinflammatory cytokines (OPN and TNFa) by adenoviral vectors is a potential and useful system to promote an efficient immune response against M. tuberculosis in an experimental model of progressive TB, being of great interest their use in MDR-TB cases.

Anti-cancer response mediated by dle transferon®-induced CD11c+ NK cells

Dalia Ramirez-Ramirez[1,2], Juan Carlos Balandrán[2,3], Marco Antonio Velasco-Velázquez[4], Rosana Pelayo[2], Sonia Mayra Perez-Tapia[1]

[1]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico
[2]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
[3]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
[4]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
dal2_r@hotmail.com; jcbalandran@gmail.com; marcovelasco@hotmail.com; rosanapelayo@gmail.com; smpt.2011@hotmail.com

Keywords: dialyzable leukocyte extract, anticancer response, CD11c+ NK cells, γδ T cells, emergent hematopoiesis

Background: Natural killer (NK) cells, the prototypic member of innate lymphoid cells, are important effectors of anticancer immune response. These cells can survey and control tumor initiation due to their capability to recognize and kill malignant cells and to regulate the adaptive immune response via cytokines and chemokines release. However, several studies have shown that tumor-infiltrating NK cells associated with advanced disease can have profound functional defects and display protumor activity. Moreover, extensive heterogeneity and plasticity of NK cell subsets may be involved and play distinct roles in the context of malignant microenvironment, including stroma and immune cells, or tumor-derived factors. NK cells have shown promise as therapy of hematological cancers. Therapies utilizing NK cells can be classified as either harnessing endogenous responses by administering NK stimulants or targeting agents or using exogenous NK cells via hematopoietic stem cell transplant (HSCT) or adoptive cell transfer (ACT) models. There are several key advantages to harnessing NK cells as part of an immunotherapy.

In our previous studies, DLE-derived NK cells endowed with properties such as IFN-y production, tumor cell cytotoxicity, and the capability of inducing gd T cells lymphocyte proliferation were shown as putative coadjuvant component of innate immune responses against virus-infected or tumor cells.

Objective: To perform molecular and functional characterization of NK CD11c+ cells induced by DLE and elucidate their contribution to anti-cancer responses in acute lymphoblastic leukemia

Materials and Methods: Umbilical cord blood (UCB) samples were obtained from normal full-term neonates upon mothers' written informed consent. CD34+ cells (HSPC) from UCB were enriched using the Human CD34 Progenitor Cell Isolation Kit; DLE Transferon is manufactured by UDIMEB at GMP facilities in the National School of Biological Sciences, National Polytechnic Institute (IPN). DLE Transferon was used for cell culture stimulation. MS-5 stromal cells were grown in presence of DLE Transferon before co-culturing with CD34+ HSPC. Upon DLE prestimulation, HSPC were cocultured in the presence of MS-5 stromal cells for 30 more days. Lymphoid lineage cytokines and growth factors were contained throughout coculture: 1 ng/mL Flt3-L (FL), 2 ng/mL SCF, 5 ng/mL IL- 7, and 10 ng/mL IL-15. Natural killer cytolytic activity was evaluated using a fluorescence-based assay, in vitro differentiated NK cells from DLE-cultures were enumerated by flow cytometry and cocultured with CTV (CellTrace Violet) REH cells (B-ALL cell line), according to an effector: target ratio curve. IL-2 was used to induce NK cell activation, followed by wash and 7-AAD incorporation. After 30-day coculture, cells were stimulated with IL-12 and IL-18 for 12 hours and NK-like cells were harvested and incubated with Brefeldin to inhibit protein intracellular transport, followed by cytometry extra- and intracellular staining, respectively. γδ T peripheral blood lymphocytes from healthy donors were enriched and stained with Cell Trace Violet (CTV). Simultaneously, newly differentiated CD11c+ NK cells from DLE-stimulated 30-day cocultures were harvested and purified by flow cytometry sorting in BD FACSAria equipment. The purified gd T cells were then cocultured with the NK cell population of interest, at a ratio of 2 : 1 CD11c+ NK: y T lymphocyte. gd T cells cells proliferation was assessed at 72 hours by dilution of CTV.

Tridimensional co-culture systems were used to determine in vitro capability of CD11c+ NK cell to colonize BM niches and their capacity to eliminate or promote the cell cycle arrest of leukemic blasts . The mesenchymal stromal cell line OP9 or MS5 was cultured in non-adherent wells, then the newly differentiated CD11c + NK cells were cocultivated for 6 h prior to REH cells addition. Migration of leukemic blasts into the spheroid and their proliferation was evaluated.

Results: The functional activity of NK cells produced from DLE-stimulated lymphoid progenitors demonstrated a high cytolytic potential and production of IFN-y from the specialized CD11c+ NK population, suggesting that early progenitors are promoted to differentiate toward both cytotoxic and regulatory cells in response to DLE. Of note, dilution of the dye CTV was significant when gd T cells cells were cultured with CD11c+NK that are produced upon stimulation with DLE. Surprisingly, neither unstimulated gd T cells cells nor DLE-directly stimulated gd T cells cells got activated.

NK CD11c + cells co-cultured with leukaemic REH cells increase in frequency and cell number when coming from the stimulation of HSPC with DLE. The profile of cytokines produced by DLE-induced NK cells in the presence of leukemic blasts predominantly corresponds to proinflammation, due to an increase in IFN-γ producing populations concomitant with the decrease in TGF-β and IL-10. Of high interest was is to observe a small population of NK IL-22 + cells that would correspond to an activated population responding to leukemic blasts.

The evaluation of proliferation of REH cells in the stromal organoid indicated that the CD11c + NK cells induced with DLE limit the colonization of the spheroid by leukemic blasts and promote the decrease in the proliferation of the few cells that they managed to enter the spheroid, in comparison to the NK cells with no extract. Of great importance is the decrease of leukemic blasts inside the organoid, suggesting their lysis by the induced NK cells.

New approach based in immunotherapy treatment of transmissible venereal tumor

Yareellys Ramos Zayas, Moisés Armides Franco Molina, Diana Ginette Zárate Triviño, Pablo Zapata Benavides, Reyes Tamez Guerra, Cristina Rodríguez Padilla

Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico
yareellysraza@hotmail.com; moyfranco@gmail.com; dianazt@gmail.com; pablozapata@hotmail.com; resitague@outlook.com; crrodrig07@gmail.com

Keywords: Dendritic Cells, Immunotherapy, Treatment, Transmissible venereal tumor, tumor exosomes

Autologous immunotherapy uses the body's own cells to stimulate and restore the natural defenses of the immune system. Some of the cells that have been used by various researchers are: tumor infiltrating lymphocytes, cytotoxic T lymphocytes, lymphocytes activated killer cells and dendritic cells (DC). DCs are specialized cells of the immune system and its role is to capture, process and present antigens to lymphocytes to initiate an immune response. A new source of antigens for DC are exosomes, small vesicles (30-120 nm) containing RNA, DNA and protein. Proteins such as tetraspanins (CD9, CD63, CD81), heat-shock protein (Hsp), major histocompatibility complex (MHC) class I and II molecules and tumour associated antigens (TAA) have been identified. They are secreted by various types of cells and found in abundance in body fluids including blood, saliva, urine, semen, tears, mucus, lymph, ascites, sweat, bronchial lavage, cerebrospinal fluid and breast milk. Exosomes are thought to function as intercellular messengers, delivering their molecules of effector or signaling between specific cells. An excellent in vivo model for immunotherapy studies is the transmissible venereal tumor (TVT), one of the four transmissible tumors in the world, is a transmissible cancer in dogs that mostly affects the external genitalia and is transmitted during sexual coition. In this work, we used DC pulsed with TVT exosomes as a treatment in canines with TVT. To study the immunotherapy three experimental groups were used: unvaccinated group; vaccinated with TVT exosomes and vaccinated with DC pulsed with TVT exosomes. For the immunotherapy a primary culture of TVT cells was performed, TVT exosomes were isolated and characterized by scanning electron microscopy assay, dot blot, and protein quantification. Then was extracted 4% of their body weight of blood of the patients to differentiate monocytes into DC in a short time protocol of 48 hours. To evaluate the effect of the immunotherapy with DC pulsed with TVT exosomes side effects were observed, also tumor size, populations of lymphocytes, IFN-γ levels in blood serum

and tumor infiltrating lymphocytes by histopathology. The TVT isolated exosomes showed a homogeneous sample with a circular shape of approximately 90 nm. In the dot blot, all the protein markers CD81, CD63, CD9, and Hsp70 were positive except the control (albumin). The protein quantification of five representative samples of the exosomes were 9.15 mg/mL, 13.65 mg/mL, 10.21 mg/mL, 11.43 mg/mL and 10.79 mg/mL. Monocytes were differentiated into DC and underwent a phenotypic analysis by flow cytometry. Monocytes showed an expression of 85.71% for CD14+, 12.3% for CD80+, 0.1% for CD83+ and 0.8% for DLA II. In DC 5.1% for CD14+, 86.7% for CD80+, 90.1% for CD83+ and 92.6% for DLA II and a phagocytosis of 63% was obtained by FITC-Dextran test. No side effect was shown in our experimental group for our therapy and the tumor regression was of 100% for the seventh week. An increase of 28% of CD4+ and 34% of CD8+ cell expression, IFN-γ level of 142 pg/mL and in the histopathology the presence of infiltrating tumor lymphocytes were found. These results showed that DC pulsed with tumor exosomes induce regression of the TVT in dogs.

Delimitation of colon cancer using modified Nidogen-1: development of an immuno-tool for tumor therapy

Gabriela Ferragut, Deborah Juana Keszenman, Juan Cedano

University of the Republic, Montevideo, Uruguay
gferragu@fq.edu.uy; dkeszen@gmail.com; jcedano@unorte.edu.uy

Keywords: Biotechnology, colorectal cancer, oncology, IL-6, Nidogen-1

Oncological surgery remains as one of the key approaches to the management of cancer despite constant innovations in other therapeutic fields such as radiotherapy, chemotherapy, and other systemic therapies that include those aimed at molecular targets. A constant challenge for surgeons is the intraoperative evaluation of tumor resection borders without prolonging surgery. The present study proposes the development and use of new biotechnological tools aimed to reduce aggressive surgical oncology treatments without compromising their therapeutic or efficacy.

To address this purpose, we aim to develop a fluorescent marker that binds to the tumor margins so as to serve as a guide for the surgeon to define as precisely as possible where the tumor ends and where the healthy tissue starts. In order to accomplish the development of this tool that will improve cancer treatments, a construct was generated from modifications to the protein nidogen-1 (picture shows the construct where mutated amino acids are depicted in gray), which is present in the extracellular matrix of human tissue.

This protein was chosen for its ability to bind to the cell matrix formation. In addition, this protein has a high structural homology with the green fluorescent protein (GFP).

Interleukin-6 is a multifunctional cytokine that has a central role in the regulation of inflammatory and immune responses. It is produced by a variety of cells and has an important role in the progression of cancer, including proliferation, migration and angiogenesis in several cancers including colorectal cancer. Since Interleukin-6 is involved in the signaling pathways participating in inflammatory and immune responses in tumors, we decided to test the colocalization a modified nidogen-1 protein with IL-6 protein, thus enabling to the proposed tool.

Methodology: The chimeric protein is formed by the amino acids of human nidogen-1 protein on the surface, and by corresponding to a protein of the family of GFP fluorescence high amino acid level inside. Due to the fact that the active site of these fluorescent proteins implies amino acids that are buried inside the molecule as well as presence of a tunnel that allows a steady flow of water molecules to the active center, we decided to transplant the full block protein inside. However, the conserved amino acids that interact with the extracellular matrix are being ensured by the same affinity. The production of the protein was carried out in Drosophila S2 cells in Schneider´s Medium Thermo Fisher Scientific.

To evaluate the efficiency of the obtained protein, a model of carcinogenesis in rats will be used that will pharmacologically induce colon tumors. After tumor induction, a surgery to stimulate tissue angiogenesis will be carried out, and thereafter we propose the administration and / or stimulation of IL-6 production.

Implications of silver nanoparticles on phenotypic changes in murine bone-marrow derived dendritic cells

Sandra Castro Gamboa[1], Sofía Gómez Bautista[1], Pablo César Ortiz Lazareno[2], Ricardo Gómez Jauregui[1], Trinidad García Iglesias[1], Maritza Roxana García García[1], Nina Bogdanchikova[3], Andres Eliu Castell Rodríguez[3]

[1]University of Guadalajara, Guadalajara, Mexico
[2]Centro de Investigación Biomédica de Occidente (CIBO), Guadalajara, Mexico
[3]Universidad Nacional Autónoma de México, México.
castrosandra11@gmail.com; sofygoba@hotmail.com; pablolazareno@gmail.com; ricaedogj100@gmail.com; trini.iglesias@gmail.com; mary_roxy13@hotmail.com; nina@cnyn.unam.mx; castell@unam.mx

Keywords: Immunomodulation, Cytotoxicity, immunophenotype, Silver nanoparticles (Ag-NPs), Bone marrow dendritic cells

Introduction: Nanotechnology is one of the great challenges of this century. The properties of nanomaterials give them a wide range of applications including new approaches in Immunology. This raises the possibility of evaluating the possible immunomodulatory effect of silver nanoparticles (AgNPs) in dendritic cells (DCs), which have been exploited by their potential for immunotherapy. Thus, in order to increase our knowledge on the immunological activity of AgNPs, it is necessary to perform research related to the interaction between AgNPs and DCs.

Objective: To analyze phenotypic changes and cytotoxicity on mouse bone marrow derived dendritic cells treated with silver nanoparticles.

Material and Methods: The bone marrow derived dendritic cells (BMDCs) were extracted from mice C57BL/6 of 6 to 8 weeks of age. The cells were differentiated from total bone marrow cells for 9 days with RPMI-1640 medium, supplemented with 10% of fetal serum bovine, and 30% transfected CHO culture supernatant with GM-CSF. The assays were done at 24 and 48 h. From a MTT assay, the most stimulating concentrations on BMDCs were selected. Whit them, it was analyzed the immunophenotype and cytotoxic effect by flow cytometry. The BMDCs population was determined by

CD11c+, and the immunophenotype with CD40, CD274, and MHC II. The BMDCs culture supernatant was analyzed by ELISA to determine the secretion of IL-6, IL-10, IL-12, and TNF-alpha.

Results: The most stimulating concentrations of AgNPs were 62.5, 125, and 250 ng/ml, with a major effect at 48 h. None of them showed a cytotoxic effect. The BMDCs population (CD11c+) was superior to 50% in all the concentrations and times. MHC II, and CD40 showed expression in a low quantity of CD11c+ cells, meanwhile in CD274 had a uniform expression at CD11c+ cells. The pro-inflammatory cytokines, IL-6, IL-12, and TNF-alpha at 24 h, were low at 24 h, but they increases at 48 h in BMDCs culture supernatant. On the other hand, the anti-inflammatory cytokine, IL-10, was higher at 24 h, and lower at 48 h.

Conclusion: Our results suggest that AgNPs could contribute to phenotypic changes in BMDCs related to immature or activated dendritic cells. This work represents the first step of the study of potential applications of AgNPs as immunomodulatory agent.

Low-immunogenic synthetic tracheal matrix, populated with mesenchymal multipotent cells

Mikhail V. Kiselevskiy[1], Natalia Yu. Anisimova[1], Olga V. Lebedinskaya[2]

[1]Russian Cancer Research Center NN Blokhin, Moscow, Russia
[2]Perm State Medical Academy, Perm, Russia
kisele@inbox.ru; n_anisimova@list.ru; lebedinska@mail.ru

Keywords: immunohistochemical study, Heterotopic implantation, Mesenchymal multipotent cells, synthetic tracheal matrix, bioartificial scaffolds

This study aims – researching the properties of the tailor-made bioartificial scaffolds promising for substitution of natural trachea with life-threatening lesions.

Synthetic tracheal scaffolds for preclinical trials on dogs were electrospun from nonwoven fabric by a unique experimental monocapillary device for electrospinning. Fluoroplastic (copolymer of tetrafluoroethylene and vinylidene fluoride) was applied as a polymer material of the nonwoven fabric ("ChimCombinat", Russia). Rings reinforcing nonwoven polymer fabric were obtained by thermopressing from polyurethane "Elastollan 1195A" (Elastogran, Germany).

Six healthy dogs ranging in weight from 15 to 22 kg were used for experiments. All animals were treated in accordance with the "Guide for the care and use of laboratory animals" prepared by the Institute of laboratory animal resources, National research Council, and published by the National Academy Press, revised 1996. The experiments applying beagle dogs were approved by the local ethical committee. General anesthesia was induced with thiopental and maintained with oxygen and 1–3% halothane through an endotracheal tube. All animals were perfused with a crystalloid solution. Pulse and arterial pressure were monitored intraoperatively.

Tracheal scaffolds were colonized by recipients' MSCs obtained from recipients' bone marrow by in vitro cultivation before the implantation for better biocompatibility and faster integration. Analysis of MSCs culture was performed using Axiovert 40 inverted microscope (Zeiss, Germany) in a transmission or phase contrast mode. Histological examination was performed by standard methods.

Sterile scaffolds colonized by MSCs were grafted into the heterotopic preformed groin intramuscular pockets. Intramuscular pockets were formed in dogs for implantation of

sample scaffolds. Scaffolds were implanted intramuscularly into femoral fossae. Surgery was conducted under general anesthesia maintaining aseptic and antiseptic conditions.

The animals were observed during a month. Surgery sites were daily visually examined. Hematological parameters were regularly monitored using an analyzer (ProCyte Dx, Netherlands). Clinical blood analysis was periodically performed to control possible development of system implant rejection or inflammatory reactions in dogs after the heterotopic grafting of the experimental samples. Hematologic characteristics remained physiologically normal in the recipient animals during a month after the implantation.

Visual monitoring did not reveal any signs of local rejection reactions: edema, suppuration, redness, fistula development, massive fibrous tissue growth at the implantation region during all the observation period.

Tracheal scaffolds extracted 1 month after the implantation preserved their form as well as their frame qualities. They were intimately linked with surrounding connective tissue capsules. Morphological examination of the implantation region did not reveal in the surrounding tissues any signs of inflammatory exudation or system leucocyte infiltration. Fibrous structure of the synthetic material was intact in the extracted scaffolds. Along with it, morphological examination revealed abundant colonization by recipient's cells, blood vessels formation and connective tissue fibers penetrating from the surface deep into the implants.

Immunohistochemical study revealed cellular composition of the scaffold surface. It was actively colonized by the recipients' connective tissue cells and by differentiating progeny of the bone marrow MSCs.

Insignificant quantities of lymphocytes were found within the synthetic scaffold. T cells (CD3+ cells) were the most abundant among the lymphocytes present in the scaffolds. Small groups of CD3-expressing cells were diffused and contained few intensively stained small and medium-sized lymphocytes.

CD68+ cells were rare; their staining was of medium intensity. This cell population includes all the macrophage-type cells. We also noticed singular CD19+ plasmacytic type cells. Interestingly, blast forms of this cell type expressed the marker on their cellular and on their nuclear membrane as well.

Limited number of Ki67+ proliferating immunocytes was present. Such cellular ratio in the bioengineered tracheal prosthesis during its heterotopic implantation in dogs evidences insignificant macrophage immune response to the implant, minimal

plasmacytic reaction and minor infiltration by singular lymphocytes. The proliferation of immune cells in the tracheal scaffold after transplantation was not active.

Thus, colonization of the synthetic tracheal scaffold by MSCs with the following heterotopic implantation allows producing tissue-engineered construction as a result of scaffold occupation by recipient's cells with formation of blood vessels and connective tissue structures. Therefore, we developing an approach to create ultrathin-fiber nonbiodegradable scaffolds, which might be colonized by recipient's cells ex vivo and in vivo, that is useful for production of tissue-engineered bioprosthesis constructions of hollow organs.

Production and characterization of monoclonal antibodies against human IgA

Miguel Angel Baños-Jiménez[1,2], Zuleima Cristina Pérez-Hernández[1,2], Leopoldo Santos-Argumedo[1], Héctor Romero-Ramírez[1]

[1]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico

[2]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico

m-banos@hotmail.com; zullmitzz@gmail.com; lesantos@cinvestav.mx; hromero@cinvestav.mx

Keywords: Antibodies, Hybridomas, IgA, mucosal immunology, monoclonal antibodies

In humans, immunoglobulin A (IgA) is the most abundant antibody in the organism, from which approximately 40 mg/kg of weight is produced in an adult per day. Humans have two subclasses of IgA (IgA1 and IgA2). IgA1 has a hinge of approximately 16 to 19 amino acids, rich in proline, serine, and threonine residues containing O-glycosylation sites. Its unusual glycosylation pattern, which differs from the rest of immunoglobulins, has been used to purify this protein with jacalin (a lectin from Artocarpus heterophyllus).

IgA1 is the predominant form in the blood, while IgA2 is more frequently distributed in mucous membranes and secretions (tears, saliva, milk, colostrum, secretions of the respiratory, gastrointestinal and urogenital tracts). IgA protects the mucous membranes of the body, being the first line of defense, neutralizing viruses and toxins, as well as preventing the adherence and invasion of bacteria and parasites, having a relevant role in mucosal immunity.

IgA is one of the antibodies produced in human milk and is essential in the passive immunity of infants against infections. Selective IgA deficiency is one of the most common primary immunodeficiencies, defined as a decrease in serum IgA levels in the presence of normal levels of other immunoglobulin isotypes. The majority of individuals with IgA deficiency are asymptomatic. However, some patients present recurrent infections in the respiratory and gastrointestinal tracts, allergic disorders and autoimmune manifestations.

The goal of this work was to obtain monoclonal antibodies to develop rapid and straightforward diagnostic methods for the detection of IgA in secretions as a tool in studies of mucosal immunity.

IgA1 was purified from human plasma by gel permeation chromatography with a stationary phase of Sephacryl S-200 and affinity chromatography with jacalin. Subsequently, a group BALB/c mouse was inoculated with 100 µg of the purified IgA1. Another group was immunized with 50 µg of IgA from human colostrum (SIGMA), using aluminum hydroxide as the adjuvant. The mice received five intraperitoneal injections within 15-day intervals in both cases. Animals with serum antibody titer higher than 1: 10,000 were used for the cell fusion.

Spleens from selected mice were extracted, and mononuclear cells were obtained to generate antibody producing hybridomas by cell fusion with the myeloma cell line (P3 / X63-Ag8-653). HAT medium was used to select hybridomas, and the production of antibodies was monitored using the ELISA technique.

We characterized two hybridomas producing monoclonal antibodies that recognized both IgA1 and IgA2. Also, four monoclonal antibodies of IgG1 isotype with kappa light chain were obtained. Those specifically reacted with the human alpha-2 chains in their native form. These reagents will be evaluated by their utility to analyze mucosal immunity in humans.

Random coil structure in immunoglobulin-like domains of CRTAM as potential regions for interaction with its ligands

Julio Angel Vázquez-Martínez[1,2], Miguel Angel Gómez-Lim[3], Jorge Alberto González-Y-Merchand[1], Vianney Ortiz-Navarrete[2]

[1]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico
[2]Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico
[3]Centro de Investigación y Estudios Avanzados, Irapuato, Mexico
bio.juliovazquez@outlook.com; miguel.gomez@cinvestav.mx; jgonzal1212@yahoo.com.mx; vortiz@cinvestav.mx

Keywords: immunoglobulin, CRTAM, Random coil, Virus-like particle (VLP), Nectin-like 2

The extracellular region of the Class I-Restricted T Cell-Associated Molecule (CRTAM) is comprised by two Ig-domains, one V-Ig-like and one C-Ig-like. The Ig-like V domain of CRTAM is shaped by eight β-sheets (A, C, C', F, G, B, E and D) separated by seven random coils, nevertheless the five sheets A, C, C', F and G are responsible to form the interface of heterotypic interaction with its ligand nectin-like 2 (Necl2) or for a homotypic contact. The random coils regions have an important role in the proteins due to have high molecular dynamic that confer flexibility and stability to secondary structure. Additionally, these structures form exposed loops that mediate the protein-protein interactions and regulate the tridimensional form of protein due to are sites of post-translational modifications.

To elucidate the biological role of CRTAM during interaction with its ligands, there are two alternatives: disrupting sterically the binding region or change the native conformation, both strategies through antibodies that recognize specific exposed regions critical to folding or for interface region. By bioinformatic tools, we found two conserved antigenic regions in human and mouse primary sequence of CRTAM. The first epitope PALKS is localized, according the crystal structure of Ig-like V domain, between C' and D sheets into the coil region structure. To localize the second epitope STERSK, we generated de novo; a tridimensional model of Ig-like C domain. It is also localized in coil region of predicted structure. To test the folding and immunogenicity of predicted epitopes, we produced two different constructs replacing "coil by coil" in the exposed region of the capsid protein L1 of human papillomavirus type 16. Both chimeric viral proteins were expressed in Nicotiana benthamiana by transitory expression system and the purified monomers showed to maintain their capacity to self-assembled into

virus-like particles (VLP). These chimeric VLP were used as carrier of epitopes to obtain polyclonal antibodies against both above-mentioned epitopes of CRTAM. We demonstrated that the sera recognized a conformational structure of extracellular region of human CRTAM fused to Fcγ region of IgG1. As well these polyclonal sera react with the native CRTAM expressed on the surface of activated CD8 T cells, meaning that structure and surface exposure of both coil region were conserved in the structure of viral capsid in similar fashion as they are exposed on CRTAM. Thus, it's likely that both regions might be important for heterotypic and homotypic interactions.

Allele diversity at six HLA LOCI: A*, B*, C*, DRB1*, DQB1*, DPB1*, in two regions of mexico determined by Sequence Based Typing (SBT) and Next Generation Sequencing (NGS): the highlands and the south-east

Andrea Munguía[1], Hilario Flores-A[1,2], Betsy A Gonzalez-Q[1,2], Guadalupe Sanchez[2], Miriam Valencia[1], Enrique Bonilla[1], Clara Gorodezky[1,2]

[1]Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), México, Mexico
[2]Fundación Comparte Vida, A.C., Mexico City, Mexico
orionica@yahho.com; hilario.flores@salud.gob.mx; bgonzalez@fundacioncompartevida.org.mx; rosasenoctubre@yahoo.com; mivama@yahoo.com.mx; enboga19@yahoo.com.mx; clarag@unam.mx

Keywords: Mexican mestizos, NGS HLA Typing, SBT for HLA, Population Immunogenetics, HLA Diversity

Introduction: In outbred populations such as Mexican Mestizos, who have different ethnic backgrounds, depending on the geographic region, the allele frequencies are fairly evenly distributed in most HLA loci with the exception of DPB1, in which only a few alleles account for the majority of the genes; these results in low levels of homozygosity, again, with the exception of DPB1, suggest overdominant selection. However HLA prevalence of each allele differs depending on the region the population comes from. The aim of this study, was to compare the HLA distribution and significance of variation in Mestizos from the Highlands Vs. those from Oaxaca, emphasizing that the ancestry is more Mediterranean and Caucasian in the former, prevailing interesting alleles of Amerindian and African origin in the Southeast.

Methods: We selected 70 Mestizos from the Highlands/Center, being 55.7% females and 44.2% males with an X age=34.6y. HLA A*, B*,C*, DRB1*,DQB1*,DPB1*, DNA genotyping was done by SBT on a 3500 ABI instrument. Allele frequency (AF) was calculated with the PyPop software; the haplotype (HF) analysis was assessed with the Arlequin 3.5.1.2 using the maximum likelihood method. Data were compared with those previously shown in 122 Mexican Mestizo individuals from Oaxaca, who were typed for 11 HLA loci by NGS for the 17W.

Results: The prevalent alleles frequency >5% for Mexican Mestizos from the Highlands are: A*02:01, *02:06, *24:02; B*35:01; *35:12; C*04:01, *06:02, *07:02; DRB1*04:07, *08:02:01; DQB1*03:02, *03:01, *04:02; DPB1*04:02:01:02, *04:01:01:01, *02:01. And

for Mexican Mestizos from Oaxaca are A*02:01:01:01, *24:02:01:01, *02:06:01:01; B*39:05:01; C*07:02:01:01; *04:01:01:01; DRB1*04:07:01, *08:02:01; DQA1*03:01:01, *04:01:01, *05:05:01:05; DQB1*03:02:01, *03:01:01:01, *04:02:01; DPA1*01:03:01:05, *01:03:01:02; DPB1*04:02:01:02, *04:01:01:01. A new allele was found: DQA1*04:01:01_E4, codon 200 C>T Leu>Leu. The 5 top of the haplotypes and their HF from the highlands are: A*02:01 C*04:01 B*35:17 DRB1*08:02 DQB1*04:02 DPB1*04:02 (0.0266); A*02:01 C*12:02 B*52:01 DRB1*15:02 DQB1*06:01 DPB1*04:01 (0.0214); A*11:01 C*12:03 B*18:01 DRB1*11:04 DQB1*03:01 DPB1*04:02 (0.0214); A*24:02 C*03:05 B*40:02 DRB1*04:07 DQB1*03:02 DPB1*04:02 (0.0214); A*26:01 C*05:01 B*44:02 DRB1*04:02 DQB1*03:02 DPB1*02:01 (0.0214). The top from Oaxaca are: A*68:03:01 B*39:05:01 C*07:02:01:01 DPA1*01:03:01:05 DPB1*04:02:01:02 DQA1*03:01:01 DQB1*03:02:01 DRB1*04:07:01 DRB4*01:03:01:01 (0.0205); A*02:01:01:01 B*39:02:02 C*07:02:01:01 DPA1*01:03:01:05 DPB1*04:02:01:02 DQA1*05:05:01:05 DQB1*03:01:01:01 DRB1*16:02:01:02 DRB5*02:02 (0.0164); A*24:02:01:01 B*39:06:02 C*07:02:01:01 DPA1*01:03:01:05 DPB1*04:02:01:02 DQA1*03:01:01 DQB1*03:01:01:01 DRB1*14:06:01 DRB4*01:03:01:01 (0.0164); A*68:03:01 B*39:05:01 C*07:02:01:01 DPA1*01:03:01:05 DPB1*04:02:01:02 DQA1*03:01:01 DQB1*03:02:01 DRB1*04:07:01 DRB4*01:03:02 (0.0164); A*02:01:01:01 B*07:02:01 C*07:02:01:03 DPA1*01:03:01:05 DPB1*04:02:01:02 DQA1*01:02:01:01 DQB1*05:02:01 DRB1*15:01:01:01 DRB5*01:01:01 (0.0123).

Conclusions: High levels of heterozygosity at all loci with exception of DP was found in Oaxaca, unique alleles and haplotipic association and finally, a new allele at DQA1 was detected.Great diversity exists in both groups, with a higher Mediterranean allele content in the highlands; 6 haplotypes found in here, are also shown in Sephardi Jews, Spaniards and Lebanese populations (Med, His, Asian, Afr, His, Asian). Several haplotypes are unique to Mexican Natives. Among the 13 main haplotypes are present, 5 exist in the Middle-East and in Sephardi Jews and 5 are unique to Mexican Natives, Afr-Am especially in those from Southern Mexico. In different groups exists the 10 haplotype. In these populations, any novel allele may have been positively selected according to their particular environment to enlarge the peptide-binding repertoire, and some are found with distinctive haplotype associations, suggesting that convergent evolution events may have also taken place. NGS is a very powerful technique to detect new alleles and have a better understanding of the nowadays features of populations.

Interesting diversity of full HLA class I and class II genes typed using NGS (Next Generation Sequencing) in the Mestizo population from the city of Oaxaca, South-East Mexico

Andrea Munguia[1], Lisa Creary[2], Marcelo Anibal Fernandez-Vina[2], Betsy A Gonzalez-Q[1,3], Hilario Flores-A[1,3], Clara Gorodezky[1,3]

[1]Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), México, Mexico
[2]Stanford University, Stanford, United States
[3]Fundación Comparte Vida, A.C., Mexico City, Mexico
orionica@yahoo.com; lcreary@stanford.edu; marcelof@stanford.edu; bgonzalez@fundacioncompartevida.org.mx; hilario.flores@salud.gob.mx; clarag@unam.mx

Keywords: MHC genes, Population Immunogenetics, NGS-Next Generation Sequencing, HLA Class I and Class II Typing, Mestizos from Oaxaca

Introduction: The need to improve the accuracy and throughput of HLA genotyping for clinical purposes, has led to the development of new HLA sequencing platforms of Next Generation Sequencing (NGS). With this novel technology, a 17% increase of the total number of alleles and 119% of the total number of alleles with complete sequences between April 2016 and April 2017 have occurred. If deep sequencing data for HLA are available, the tailing of overlapped reads provides phase information and thus HLA allele sequences, but automatic data analysis gives bright prospect for achieving allele level genotyping. These studies have revealed an heterogeneous combination of Amerindian, Caucasian, and African genes in Mexican admixed individuals depending on the geographical region. The City of Oaxaca, was founded in 1486 by Aztec warriors, and the population results nowadays, from the admixture of the Spaniards and of 18 Native local groups, mainly Mixteco and Zapoteco. An important group of African slavers brought by the Spaniards tried to subdue the local Natives. We selected 104 registered donors of the city of Oaxaca.

Methods: DNA samples were extracted from peripheral blood with the Maxwell 16 instrument. HLA typing was achieved by amplification by long range PCR with full gene coverage for class I loci and large coverage of class II loci including all exons with exception of exon-2. All amplicons for each sample were pooled and tagged for sequencing and sample identification; 24 samples at a time were pooled and sequenced with an Illumina Miseq instrument. Analyses of sequences was determined with Mia Fora software by the application of ad-hoc develop programs that utilized three logic

components including mapping of sequences to references and by performing full assembly. With exception of DPB1, genotypes at all loci, were assigned unambiguously. Allele frequencies (AF) were assessed with the PyPop software and the haplotype (HF) analyses was done with the Hapl-o-Mat v.1.1 software.

Results: The results showed that the prevalent alleles (AF>10%) are: A*02:01:01:01, *24:02:01:01, *02:06:01:01; B*39:05:01; C*07:02:01:01; *04:01:01:01; DRB1*04:07:01, *08:02:01; DQA1*03:01:01, *04:01:01, *05:05:01:05; DQB1*03:02:01, *03:01:01:01, *04:02:01; DPA1*01:03:01:05, *01:03:01:02; DPB1*04:02:01:02, *04:01:01:01. A new allele was found: DQA1*04:01:01_E4, codon 200 C>T Leu>Leu. The four prevalent haplotypes were: A*68:03:01, B*39:05:01, C*07:02:01:01, DPA1*01:03:01:05, DPB1*04:02:01:02, DQA1*03:01:01, DQB1*03:02:01, DRB1*04:07:01, DRB4*01:03:01:01 (HF=0.0205); A*02:01:01:01, B*39:02:02, C*07:02:01:01, DPA1*01:03:01:05, DPB1*04:02:01:02, DQA1*05:05:01:05, DQB1*03:01:01:01, DRB1*16:02:01:02, DRB5*02:02 (HF=0.0164); A*24:02:01:01, B*39:06:02, C*07:02:01:01, DPA1*01:03:01:05, DPB1*04:02:01:02, DQA1*03:01:01, DQB1*03:01:01:01, DRB1*14:06:01, DRB4*01:03:01:01 (HF=0.0164); A*68:03:01, B*39:05:01, C*07:02:01:01, DPA1*01:03:01:05, DPB1*04:02:01:02, DQA1*03:01:01, DQB1*03:02:01, DRB1*04:07:01, DRB4*01:03:02 (HF=0.0164).

Conclusions: This very subtle technology allowed us to demonstrate additional level of diversity identified at non-coding segments. We also showed high levels of heterozygosity at all loci with exception of DPA1 and DPB1. Unique alleles and haplotypic associations were detected in Mexican Mestizos and a new allele at DQA1 was found. Interestingly, predominant Native alleles not observed in other ethnic groups, were found in this populations, and haplotypes differentiating at multiple loci. NGS is also a marvelous tool for identifying genetic events such as allele lineage diversification, recombination and convergent evolution. Finally, NGS allow us to characterize more than one common allele associated with the same protein and intron variation appears to be haplotype Specific. These, and similar data, are very useful for applications that depend on allele prevalence information: the reduction and resolution of ambiguity for research and clinical practice, the identification of potential matches for hematopoietic stem cell transplantation and the general management of the general data in the face of an ever growing list of recognized allele sequences.

Polymorphism analysis of 24 Short Tandem Repeats (STRs) in a large sample of healthy mexican mestizo subjects studied for paternity testing

Hilario Flores-A[1,2], Araceli Rodríguez[1], Betsy A Gonzalez-Q[1,2], Andrea Munguía[1], Clara Gorodezky[1,2]

[1]Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), México, Mexico
[2]Fundación Comparte Vida, A.C., Mexico City, Mexico
hilario.flores@salud.gob.mx; chelinda69@yahoo.com.mx; bgonzalez@fundacioncompartevida.org.mx; orionica@yahoo.com; clarag@unam.mx

Keywords: Chimerism, Population Genetics, Paternity testing, Mexican mestizos, STRs-Shirt Tandem Reoeats

Introduction: STRs are versatile and informative genetic markers that have facilitated a great understanding of the genetic diversity present in natural populations with patterns of genetic variation within and between populations have provided novel insights into the origin and history of humans, being a common tool in forensics, paternity and anthropological studies. Data collection for such studies is conducted based on many groups, allele frequency (AF) must be established in making an identification or any study. Mexican Mestizos are of mixed descent and are the result of admixture between the Indigenous people of Asian ancestry inhabiting the country, different Europeans, and to a lesser extent, Africans. We intended to describe the pattern of 24 STRs in Mexican Mestizos for the above purposes.

Methods: STRs were typed in 817 Mestizo adults, born in Mexico, (475 males (58.1%) and 342 females (41.8%) by using PowerPlex21, GenePrint 24 (Promega), Global filer and Identifiler (Thermofisher Scientific Sci.) systems. We investigated 24 autosomal STRs in genomic DNAs. The STRs were run in a 3500 Sequencer and the DNA fragment analysis was done with the Gene Mapper ID-X v1.4 software. Allele frequency (AF) was compared with other studies done for forensics. Linkage disequilibrium(Δ), hardy-Weinberg equilibrium (HWE), Power of Exclusion (PE), Power of Discrimination (PD) were determined were estimated with the PowerStat software and R Pearson value was use compared the frequency with other studies done in Mexicans from different regions.

Results: A total of 349 alleles at the 24 STR loci were found with corresponding AF ranging from 0.061-52.39. STRs are highly polymorphic being the most diverse FGA,

D18S51, PentaE, D21S11, SE33 and D6S1043 (31-18 alleles). The most frequent alleles >25% were; 10-D2S441; 11-D5S818; 11-D7S820; 14- D6S1043; 14-D19S433; 19 & 20-D12S391; 10-D7S820. These were compared with data of Central Mexico and from 13 CODIS from Latin American and Caribbeans. LD displayed no association between paired loci. Our data are concordant with those shown in Monterrey (North Mexico) but show different AF from them & those found in other Mexico regions. The most frequent alleles >20% were at; D21S11; D6S1043; vWA;TH01;CSF1P0; D16S539; D8S1179; D3S1358; D19S433, D2S391; D7S820.

Conclusions: The 24 autosomal STR loci typed herein, provide highly informative polymorphic data, for population genetics, paternity testing and forensic identification; since the R Pearson value= 0.985 (SD=0.02). Some alleles were in Δ, meaning they are not in random association in 13/24 STRs (D16S539, D12S391, D19S433, PentaD, FGA, TH01, TPOX, D18S51, PentaE, D21S11, D18S51, D3S1358, CSF1PO). The PD=0.95, shows high heterozygosity and low probability of finding two individuals with the same genotypes and PE=0.947 shows the power of detecting variation (Table 2). STRs may be used together, since many are in Δ, to solve deficient kinship cases or mutations. Because of their high diversity, they can be applied in the identifications of some special paternity testing cases. They are also very informative for chimerism studies after hematopoietic stem cell transplantation.

ALTHEA Gold Libraries™: novel antibody libraries for therapeutic antibody discovery

Philippe Valadon[1], Sonia Mayra Perez Tapia[2], Renae S. Nelson[1], Omar Uriel Guzmán Bringas[2], Hugo Ivan Arrieta Oliva[2], Keyla M. Gómez Castellano[2], Mary Ann Pohl[3], Juan C. Almagro[4]

[1]Antibody Design Labs, San Diego, United States
[2]Unidad de Desarrollo e Investigación en Bioprocesos, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico, Mexico
[3]Tri-Institutional Therapeutics Discovery Institute, New York, United States
[4]GlobalBio Inc, Cambridge, United States
pvaladon@gmail.com; smpt.2011@hotmail.com; Juan.C.Almagro@globalbioinc.com; uriel-gbringas@hotmail.com; hugo.oliva67@gmail.com; keylamgomez@gmail.com; mapohl@tritdi.org; jcalmagro@hotmail.com

Keywords: Protein model, semisynthetic scFv libraries, peptide targets, specific antibody fragments, Therapeutic Antibody Discovery.

We present here, the design, implementation and validation of ALTHEA (from the Greek "to heal") Gold Libraries™. ALTHEA Gold Libraries™ are semisynthetic scFv libraries built on synthetic human well-known VH and VL germline genes combined with natural H3J fragments obtained from a pool of 200 human donors. The three germline genes provide a universal VH scaffold paired with two distinct VL scaffolds. The VL scaffolds offer two alternative antigen-binding site topographies to bind protein or peptide targets. The antigen-binding site (except the CDR-H3) diversity is designed in positions identified as in contact with protein and peptide antigens in the known antigen-antibody complex structures. The diversification regimes are designed based on the amino acid frequencies of the repertoire of human germline genes and known human antibody sequences. Through a proprietary process comprising the selection for thermostable scFv variants, followed by shuffling of the natural H3J fragments, ALTHEA Gold Libraries™ provide a highly diverse, functional and developable repertoire of human antibody fragments suitable for antibody therapeutic discovery and development. The potential of ALTHEA Gold Libraries™ to generate specific antibody fragments has been assessed with four targets including a protein model (Hen Egg White Lysozyme; HEL) and three undisclosed therapeutic targets. In all the four case studies, diverse and specific antibodies were obtained. A Rapid Affinity Maturation (RAM) kit™ for facile affinity maturation of selected scFvs or intermediary pools of scFvs after a few rounds of selection is also presented.

Cortactin is highly expressed in B-cell precursor cells of childhood acute lymphoblastic leukemia: implications for transendothelial migration and relapse

Martha Velázquez[1,2], Juan Carlos Balandrán[1,2], Dalia Ramírez[1], Mirella Velázquez[3], Antonio Sandoval[4], David Salomon Dozal[4], Briceida López[3], Michael Schnoor[2], Rosana Pelayo[1,5]

[1]*Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico*
[2]*Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México, Mexico*
[3]*Hospital Infantil de México Federico Gómez, Mexico City, Mexico*
[4]*Instituto Materno Infantil del Estado de México (IMIEM), Toluca, Mexico*
[5]*Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico*
mvelazquez016@gmail.com; jcbalandran@gmail.com; dal2_r@hotmail.com; mirellavelazquez@hotmail.com; sandoval.mx@gmail.com; david_dozal@hotmail.com; brisalm@yahoo.com.mx; mschnoor@cinvestav.mx; rosanapelayo@gmail.com

Keywords: Cortactin, relapse, Infiltration, Acute Lymphoblastic Leukemia (ALL), Tranendothelial migration, Actin Binding Protein (ABP)

Background: Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer disease worldwide, whose main complication is relapse, and infiltration to the central nervous system (CNS), testis, liver or lung by precursor leukemic cells. Leukemia relapse preferentially occurs in the bone marrow (BM), although other organs are also sometimes affected. However, the mechanisms of extramedullary dissemination of leukemic cells is not completely understood, but factors that drive cell movement, adhesion and transendothelial migration (TEM) of normal leukocytes are involved. These events require dynamic remodeling of the actin filament network in response to a chemoattractant gradient orchestrated by actin-binding proteins. Cortactin and its homologue HS1 are actin-binding proteins involved in adhesion and migration in virtually all cell types. While cortactin overexpression is associated with invasiveness of a number of solid tumor cells, HS1 is correlated with poor prognosis of chronic leukemia in adult patients. However, the role of cortactin in the migration of ALL-initiating cells and infiltration capabilities has never been studied.

Objective: To determine the expression and functional implication of cortactin in transendothelial migration of ALL-initiating cells and infiltration.

Materials and Methods: B-Acute lymphoblastic leukemia (B-ALL) cell lines REH and RS4:11 were used, as well as B-ALL mononuclear cells from bone marrow specimens of pediatric patients. Mononuclear cells from umbilical cord blood (UCB) were used as controls.

BM aspiration and cerebrospinal fluid (CSF) samples were collected from children and adolescents younger than 18 years that were newly diagnosed with B-ALL before starting any treatment, or upon relapse.

RNA was isolated from REH cells and CD19+ cells sorted from UCB; and cDNA was synthetized to determine gene transcript levels of cortactin by quantitative RT-PCR and the ΔΔCT method.

To determine protein levels, cell lines and mononuclear cells were stained using anti-CD34, anti-CD19 and anti-cortactin, and analyzed by flow cytometry. Cell lines and precursor cells from B-ALL patients were permeabilized and fixed for immunofluorescence staining with anti-cortactin, anti-HS1 and phalloidin to analyze the localization of these proteins.

To investigate the capability for transendothelial migration (TEM) of leukemic cells, a monolayer of HUVEC was seeded on filters with pores of 5 μm and leukemic cell lines were placed on top and allowed to transmigrate for 4 hours using CXCL12 as chemoattractant. Subsequently, cells on the top and in the bottom wells were permeabilized, fixed and stained with anti-cortactin and analyzed by flow cytometry.

Tridimensional co-culture systems were used to determine in vitro the capability of leukemic cells to colonize BM niches and its relationship with cortactin levels. The mesenchymal stromal cell line OP9 was cultivated in non-adhesion wells, then primary cells or cell lines were co-cultured for 24 h and cortactin levels were compared between colonizing cells and and cells that remained outside the stromal spheroid.

REH cell line and primary B-ALL cells were injected into NSG mice via the vein tail, and once disease was established, mice were euthanized and BM, liver, spleen, brain, lung and testis were harvested, digested and analyzed by flow cytometry.

Results: We found substantially higher levels of cortactin mRNA in the B-ALL cell line REH isolated from a bone marrow relapse patient, when compared to normal counterparts; and it correlated with high cortactin protein levels. Then, we compared cortactin expression of REH with RS4:11, another B-ALL cell line, and observed that

RS4:11 express significantly less cortactin compared to REH. Higher cortactin expression levels in REH cell correlated with a more aggressive phenotype of REH cells.

We showed that cortactin is localized preferentially at the cell edge and co-localized with its homologue HS1 and F-actin suggesting an involvement in actin-dependent cellular processes such as adhesion and migration.

To study whether cortactin is involved in transendothelial migration of B-ALL precursor cells, we performed transendothelial migration assays in vitro of REH and RS4:11 cells with CXCL12 as chemoattractant. Analysis of cortactin expression in cells that transmigrated after 4 hours from the bottom chambers of the transwell filters versus non-transmigrated cells from the top of the endothelial monolayer revealed that transmigrated cells had significantly higher levels of cortactin in both cell lines. Considering the differences in cortactin expression levels in REH and RS4:11 cells, we performed a competitive transmigration assay with both cells in a 1:1 relation and observed that REH cells (cortactin-high) transmigrated significantly more than RS4:11 cells (cortactin-low).

Next, we analyzed primary B-ALL cell populations from bone marrow to identify whether progenitor, precursor Pro-B or precursor Pre-B populations show different cortactin expression. We observed high but heterogeneous cortactin expression among patients and there was no significant correlation of cortactin expression levels with a specific population. However, when we included a group of relapse patients, they notably showed highest cortactin expression in all populations (progenitors, Pro-B and Pre-B). Relapse BM cells had 10-fold increased cortactin levels compared to their normal counterparts, and an 8-fold increase was detected when compared to non-relapsed ALL patients. Moreover, failure to respond to steroids was also significantly correlated with high cortactin levels.

Given the relationship of high cortactin levels and BM relapse, we analyzed the capability of leukemic cells to colonize bone marrow in vitro using a 3D system of bone marrow stromal cells previously established by our group. We found indeed that B-ALL cells with high cortactin levels had an advantage in colonizing hypoxic hematopoietic niches suggesting that cortactin is a key element in the positioning of primitive cells within specialized niches.

Additionally, we showed that leukemic cells that infiltrated the cerebral spinal fluid of B-ALL patients were always positive for cortactin indicating that cortactin is involved in the infiltration to extramedullary organs. This was proven by xenotransplantion

experiments using immunodeficient NSG mice with REH or with primary B-ALL cells, which revealed that leukemic cells that infiltrated brain, lung and testis expressed the highest cortactin levels.

Conclusion: Cortactin is over-expressed in B-ALL cells and provides a transmigratory advantage triggering extramedullary infiltration and relapse.

Production, selection and characterization of monoclonal antibodies against outer membrane vesicles derived from Bordetella pertussis

Maia Lina Elizagaray[1], Daniela Flavia Hozbor[2], Martin Rumbo[1], Griselda Noemi Moreno[1]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]CONICET Instituto de Biotecnologia y Biologia Molecular (IBBM), La Plata, Argentina
elizagaraym@gmail.com; hozbor.daniela@gmail.com; rumbo.martin@gmail.com; griselda.moreno@gmail.com

Keywords: Vaccine, monoclonal antibodies, outer membrane vesicles, Pertussis, anti GroEL

Outer-membrane vesicles (OMVs) are naturally non-replicating spherical nanoparticles derived from Gram-negative bacteria. OMVs are highly immunogenic and consist of phospholipids, LPS, outer membrane proteins and entrapped periplasmic components (Ellis et al., 2010; Hozbor et al., 1999; Ormazábal et al., 2014). These vesicles participate in different biological functions such as cell to cell communication, surface modifications and host-pathogen interaction by shuttling an array of virulence factors from bacteria to host during infection (Ellis and Kuehn, 2010). Because of their immunogenic properties, self-adjuvanticity and ability to be taken up by mammalian cells, OMVs are attractive candidates for vaccine delivery platforms. We developed a new vaccine against pertussis based on OMVs, which have been shown to be safe and to induce protection in mice, with a mixed Th1/Th17/Th2 profile, a robust antibody response and adequate protection capacity against different B. pertussis genetic background including those not expressing the virulence factor pertactin (PRN) that is also a vaccine antigen (Asensio et al., 2011; Bottero et al., 2016; Gaillard et al., 2014; Roberts et al., 2008). We have characterized the composition of the pertussis nanoparticles, finding the presence of a high number of immunogens in the vaccine formulation, being an important feature since this may avoid the excessive selective pressure conferred by a single or a few protective vaccine antigens. It is estimated that the final cost per dose is less than that of existing acellular formulations based on several purified protein immunogens, which impacts on the final cost of the vaccine, which is important for its application in developing countries. In the present work we present the results obtained during the generation and characterization of anti-OMVBp monoclonal antibodies (MAbs). At the moment the production of monoclonal antibody (Mab) for the OMVBp has not been described. For the generation of MAbs, BALB/c mice were immunized three times by intraperitoneal injections (separated every 25 days) with 30 μg of OMV from B. pertussis with adjuvant. Monoclonal antibodies were derived by somatic cell hybridization as described by Galfré and Milstein (Galfrè and

Milstein, 1981) using polyethylene glycol (MW 3350) as fusogenic agent. Briefly, NS0 myeloma cells were fused with spleen cells from immunized mice and the resulting hybridomas were cloned by limiting dilution. Specific reactivity of supernatant was tested by ELISA with protein lysate of B pertussis as antigen. As first screening, 30 positive clones were selected. Upon expansion and analysis of reactivity pattern by immunoblots, most of them showed reactivity mainly directed to chaperone Gro-EL, a conserved protein, found in many species of bacteria as well as in eukaryotic cells. Interestingly, anti-Gro-EL antibodies were detected after human pertussis vaccination however when the purified non-native protein was used as vaccine little protective capacity was observed (Burns et al., 1991). In the present work we assessed whether the antibodies induced by the native Gro-EL protein contained in the OMVs were able to induce protection against pertussis infection in the mice model. To this end and to obtain high levels of monoclonal antibody, hybridoma cells selected were injected intraperitoneally into mice to induce formation of ascites tumours and production of ascites fluid containing higher levels of monoclonal antibodies. Passive transfer assay was performed with MAb24G12H8 (IgG2a). Sera (100 µl) from OMVBp immunized mice were used as positive control. Challenge with sublethal dose (1.106 CFU) of BpTohama strain was performed 24 h after transfer and protection was assessed by determining CFU counts 7 days after challenge. The results expressed as log CFU/lungs (5.8±0.2 for PBS group, 6.1±0.2 for MAb group and 3.3±0.3 for positive control) showed that there was no protection for the case of MAb24G12H8 and it remains to be determined for the case of MAb52F2B9. In conclusion we were able to obtain OMV-specific mAbs with high yield that though does not present protective capacity they constitute a valuable immunochemical tool that could be used in future different assays including quality control test for the process of obtaining the OMVs.

REFERENCES

Asensio, C. J. A., Gaillard, M. E., Moreno, G., Bottero, D., Zurita, E., Rumbo, M., et al. (2011). Outer membrane vesicles obtained from Bordetella pertussis Tohama expressing the lipid a deacylase PagL as a novel acellular vaccine candidate. Vaccine 29, 1649–1656. doi:10.1016/j.vaccine.2010.12.068.

Bottero, D., Gaillard, M. E., Zurita, E., Moreno, G., Martinez, D. S., Bartel, E., et al. (2016). Characterization of the immune response induced by pertussis OMVs-based vaccine. Vaccine 34, 3303–3309. doi:10.1016/j.vaccine.2016.04.079.

Burns, D. L., Gould-Kostka, J. L., Kessel, M., and Arciniega, J. L. (1991). Purification and immunological characterization of a GroEL-like protein from Bordetella pertussis. Infect. Immun. 59, 1417–22.

Ellis, T. N., and Kuehn, M. J. (2010). Virulence and immunomodulatory roles of bacterial outer membrane vesicles. Microbiol. Mol. Biol. Rev. 74, 81–94. doi:10.1128/MMBR.00031-09.

Ellis, T. N., Leiman, S. A., and Kuehn, M. J. (2010). Naturally produced outer membrane vesicles from Pseudomonas aeruginosa elicit a potent innate immune response via combined sensing of both lipopolysaccharide and protein components. Infect. Immun. 78, 3822–31. doi:10.1128/IAI.00433-10.

Gaillard, M. E., Bottero, D., Errea, A., Ormazábal, M., Zurita, M. E., Moreno, G., et al. (2014). Acellular pertussis vaccine based on outer membrane vesicles capable of conferring both long-lasting immunity and protection against different strain genotypes. Vaccine 32, 931–937. doi:10.1016/j.vaccine.2013.12.048.

Galfrè, G., and Milstein, C. (1981). Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol. 73, 3–46.

Hozbor, D., Rodriguez, M. E., Fernández, J., Lagares, A., Guiso, N., and Yantorno, O. (1999). Release of outer membrane vesicles from Bordetella pertussis. Curr. Microbiol. 38, 273–8.

Ormazábal, M., Bartel, E., Gaillard, M. E., Bottero, D., Errea, A., Zurita, M. E., et al. (2014). Characterization of the key antigenic components of pertussis vaccine based on outer membrane vesicles. Vaccine 32, 6084–6090. doi:10.1016/j.vaccine.2014.08.084.

Roberts, R., Moreno, G., Bottero, D., Gaillard, M. E., Fingermann, M., Graieb, A., et al. (2008). Outer membrane vesicles as acellular vaccine against pertussis. Vaccine 26, 4639–4646. doi:10.1016/j.vaccine.2008.07.004.

Il-33 and the pro-inflammatory response of colonic fibroblasts from patients with inflammatory bowel disease are modulated by interleukin-17

Renata Curciarello[1], Federico Pérez[1], Ana Sol Roldán[1], Karen Dubois Camacho[2], Santiago Brayer[3], Alicia Sambuelli[3], Gustavo Javier Correa[4], Martín Yantorno[4], Fernando G Chirdo[1], Marcela A. Hermoso[2], Thomas T MacDonald[5], Guillermo Horacio Docena[1]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]Instituto de Ciencias Biomédicas, Universidad de Chile, Santiago, Chile
[3]Hospital Udaondo, Buenos Aires, Argentina
[4]Hospital Interzonal General De Agudos General San Martín, Argentina
[5]Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom
renata.curciarello@gmail.com; federico271991@gmail.com; anasolroldan92@gmail.com; karenduboisc@gmail.com; santiagobrayer@gmail.com; alicia.sambuelli@gmail.com; gustavocorrea27@hotmail.com; martinyantorno@hotmail.com; fchirdo@biol.unlp.edu.ar; marcehr@gmail.com; t.t.macdonald@qmul.ac.uk; guidoc@biol.unlp.edu.ar

Keywords: Fibrosis, inflammatory bowel disease, ulcerative colitis, Crohn's Disease, IL-17, IL-33, Human Colonic Myofibroblasts

Inflammatory bowel diseases (IBD), mainly Crohn's disease (CD) and ulcerative colitis (UC), are chronic intestinal disorders caused by environmental and genetic factors. The gut-associated immune system is permanently activated in IBD, leading to an increase of pro-inflammatory cytokines, mediating immune cell activation/proliferation and extracellular matrix remodeling processes that cause the intestinal lesions (abscesses, fistulae, fibrosis and stenosis). Even though it is widely known that cytokines are involved in the fibrotic process, currently no anti-inflammatory therapy is available to modulate or reverse fibrosis.

Th17 cells and IL-17 are abundantly found in the inflamed intestinal mucosa, although IL-17A has been reported as a mediator of IBD, its exact role remains unclear. We have previously shown that IL-17 dimmers (IL-17AA, FF and AF) are differentially produced by UC and CD lamina propria cells, and even the anti-inflammatory properties of IL-17AA. Additionally, IL-33 and its receptor ST2 are increased in inflamed mucosa from UC patients and in a lesser extent, from CD patients. We have found that activated intestinal myofibroblasts trigger the ulcerative lesions in UC, but not in CD, while secreting IL-33, and thus supporting a functional role for IL-33 in ulceration and wound healing in UC.

In order to identify target cells for IL-17 in IBD intestinal mucosa, we studied the effect of IL-17AA, IL-17FF and IL-17A/F on the inflammatory response of human colonic myofibroblasts. Additionally, investigations were carried out on the modulation of the IL-33/ST2 axis by IL-17.

Myofibroblasts isolated from intestinal biopsies/surgical samples from adult patients (UC n=3, CD n=4) and healthy donors (HC n=1) were found to secrete IL-6 and IL-8 (detected by ELISA) when stimulated for 24 hours with recombinant human IL-17A, IL-17F or IL-17AF (1ng/ml) (combined with 1ng/ml TNF or medium). Moreover, increased expression of IL-33 and ST2 was detected in myofibroblasts exposed to similar conditions (qPCR and confocal microscopy).

Further analysis of myofibroblasts stimulated with IL-17 dimmers revealed no secretion of IL-6 or IL-8, however IL-17A+TNF diminished cytokine production compared with TNF on its own ($p<0.1$). Lastly, IL-17 dimmers induced a two- to eight-fold increase in IL-33 and ST2 expression on UC and CD fibroblasts at both mRNA and protein levels (expression levels were higher in CD fibroblasts from inflamed than uninflamed mucosa from the same patient ($p<0.05$)).

In conclusion, we found that intestinal myofibroblasts from IBD patients are target cells for IL-17 and IL-17 dimmers differentially modulated the pro-inflammatory response. Moreover, CD fibroblasts expressed IL-33 and ST2, which has been described so far as exclusively produced by UC fibroblasts. Understanding the role of cytokines involved in IBD mucosal inflammation may pave the way to identify new therapeutic targets to prevent or control the IBD-associated fibrosis process.

Immunomodulatory effects of a third generation synthetic nitroalkene in solid organ allotransplantation

Germán Galliussi[1,2], Claire Usal[3], Lucía Colella[2,4], Williams Porcal[2,4], Marcelo Hill[1,2,5], Ignacio Anegon[3], Gloria Virginia López[2,4], María Cristina Cuturi[3], Carlos Batthyány[1,2], Mercedes Segovia[1,2,5]

[1]FOCIS Centre of Excellence, Montevideo, Uruguay
[2]Institut Pasteur de Montevideo, Montevideo, Uruguay
[3]INSERM UMR1064 Centre de Recherche en Transplantation et Immunologie, Nantes, France
[4]Facultad de Química, Universidad de la República, Montevideo, Uruguay
[5]Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
ggalliussi@gmail.com; claire.usal@40univ-nantes.fr; lucia_c88@hotmail.com; wporcal@gmail.com; hillmongabure@gmail.com; ignacio.anegon@univ-nantes.fr; gvlguy1970@gmail.com; Maria-Cristina.Cuturi@univ-nantes.fr; carlos.batthyany@gmail.com; msegovia@pasteur.edu.uy

Keywords: Immunomodulation, allograft rejection, NLRP3 inflammasome, nitroalkenes, Therapy for transplantation

Organ or tissue transplantation is currently the only definitive solution when a vital organ has total failure of its functions. In clinical practice allografts are necessarily treated with chronic immunosuppression to avoid acute rejection. However, chronic rejection has not yet been successfully managed. In addition, the side effects associated with the chronic use of immunosupressants constitute a therapeutic problem due to their toxicity and global immunosuppression of the patient. New therapeutic strategies are needed to avoid their chronic use and high doses of them. Until now, all therapies to avoid the rejection of allotransplantation had as objective the manipulation of the adaptive immunity. However, it has been suggested that the inflammasome inhibition could be a strategy to generate active mechanisms to protect the graft. Our work aims to study the effect of inflammasome activation in the alloresponse and to develop a therapeutic approach based on the pharmacological inflammasome inhibition with a new anti-inflammatory nitroalkene (EP2016). It was designed and synthesized in our laboratory. In vitro, EP2016 inhibits NF-kappaB and the NLRP3 inflammasome-dependent IL-1beta release, activates NRF2/Keap1 pathway that leads to the expression of HO-1, a vascular protective enzyme. In vivo, EP2016 is able to inhibit IL-1beta secretion in the LPS-challenge model that implies the intraperitoneal injection of LPS. Moreover, in a minor histocompatibility antigen mismatched skin allograft model, mice treated only with EP2016 showed significantly prolonged allograft survival vs. the group treated only with vehicle (control). To evaluate the inflammasome role in the alloresponse, we have preliminary results in vivo comparing allograft survival in

mice knockout for proteins involved in the inflammasome pathway. In Caspase-1/11 Knockout (KO) mice allograft survival trend was prolonged vs. wild type, while in Tmem176b KO allograft survival did not change. Also, EP2016 was studied in a rat model of heterotopic heart allograft fully MHC mismatched, in which it was capable to prolong the heart grafted survival respect to the control and its precursor drug. In summary, our results show that NLRP3 inflammasome activation is a mechanism that mediates allotransplantation rejection and therefore constitutes a possible new immune checkpoint as therapeutic target. To this end, we generated a new drug, EP2016, which inhibits the inflammasome in vitro and prolongs allograft survival in vivo. Our perspectives are to develop a novel therapeutic protocol, which will include the combination of EP2016 with classical immunosuppressants (administered in low doses and short duration) in order to obtain synergistic and long-term effects.

Siglec-1 promotes the activation of human neutrophils

Ismael Secundino[1], Angel Flores-Alcantar[1], Victor Nizet[2], Yvonne Rosenstein[1,3]

[1]National Autonomous University of Mexico, Cuernavaca, Mexico
[2]University of California, San Diego, San Diego, United States
[3]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
isv@ibt.unam.mx; alcantar@ibt.unam.mx; vnizet@ucsd.edu; yvonne@ibt.unam.mx

Keywords: Neutrophils, Reactive Oxygen Species, Sialic Acids, Siglecs, CD169

Sialic acid-binding Ig-like lectins (Siglecs) are signaling receptors that modulate leukocyte immune responses. Siglec-1 (also known as CD169) recognizes glycoproteins that possess terminal sialic acids with α2,3 linkage to the underlying galactose. Activation of T cells upregulates the expression of sialylated ligands that interact with Siglec-1, promoting lymphocyte cell death. A recombinant protein consisting of the first four-Ig-like domains fused to the Fc region of human IgG1 (Siglec-1–Fc) is known to bind predominantly to granulocytes, monocytes, lymphocytes, and to a lesser extent, NK cells.

Neutrophils are the most abundant circulating white blood cells in humans, representing 50-70% of bloodstream leukocytes, and are "first responders" to tissue foci of infection to clear bacterial pathogens by phagocytosis, production of reactive oxygen species (ROS), and/or deployment of neutrophil extracellular traps (NETs). Here, we report for the first time that Siglec-1–Fc, but not Siglec-6–Fc or Siglec-9–Fc, bound to human neutrophils. Furthermore, recognition of neutrophils by Siglec-1-Fc induced expression of activation markers and production of ROS. We discuss the relevance of priming the neutrophil response via recognition of Siglec-1 during a bacterial infection model.

Role of CD43 in the establishment of the immune microenvironment during Mycobacterium tuberculosis infection

Estefanía Alemán Navarro[1,2,3], Álvaro Torres Huerta[1,2], Ángel Francisco Flores Alcantar[1,2], Cristina Parada Colín[4], Dulce Adriana Mata Espinosa[5], Clara Espitia[4], Rogelio Hernández Pando[5], Yvonne Rosenstein[1,2]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico
[2]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
[3]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[4]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[5]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
steph@ibt.unam.mx; avery@ibt.unam.mx; alcantar@ibt.unam.mx; cristinaparada2004@yahoo.com.mx; dulce.mata@quetzal.innsz.mx; espitia@biomedicas.unam.mx; rhpando@quetzal.innsz.mx; yvonne@ibt.unam.mx

Keywords: Cytokines, Macrophages, Mycobacterium tuberculosis, DnaK, CD43, Cpn60.2, BCG Bacillus Calmette Guerin

Mycobacterium tuberculosis is the causative agent of tuberculosis, which is a major public health problem worldwide. According to the last report of the World Health Organization, this disease is the first cause of death due to an infectious agent on a global scale. The only available vaccine against tuberculosis is Bacille Calmette-Guérin (BCG), an attenuated strain of Mycobacterium bovis. BCG prevents tuberculous meningitis and miliary tuberculosis during childhood, but it does not confer total protection against pulmonary tuberculosis in adults. The lack of an effective vaccine, along with the emergence and persistence of multidrug-resistant strains of M. tuberculosis, makes the eradication of tuberculosis particularly difficult. Developing new strategies to fight tuberculosis efficiently requires an increasingly detailed characterization of the infection process and the resulting immune response at the cellular and molecular level.

A critical piece to understand the pathogenesis and outcome of the M. tuberculosis infection is the study of the interactions between M. tuberculosis ligands and their matching receptors in the mycobacterium´s main target cell, the macrophage. One of the macrophage receptors that has been recently reported to interact with M. tuberculosis is the sialomucin CD43, an abundantly expressed type I transmembranal protein. Particularly, CD43 interacts with Cpn60.2 and DnaK, two molecular chaperones localized in the mycobacterial capsule. Both chaperones are critical for M. tuberculosis, as

Cpn60.2 is essential for its viability and DnaK is needed for its growth. Cpn60.2 can also function as an adhesin, mediating the macrophage - mycobacteria interaction.

Our research aims to characterize the contribution of CD43 to the immune microenvironment of human and murine macrophages stimulated with Cpn60.2, DnaK, BCG or M. tuberculosis. Overall, our results indicate that CD43 promotes the production of a complex combination of cytokines in the macrophage-like cell line THP-1 and bone marrow-derived murine macrophages in response to these stimuli. Our data also suggest that CD43 influences the bactericidal capability of THP-1 macrophages exposed to M. tuberculosis. As work done with murine models has shown that, upon M. tuberculosis infection, the lack of CD43 disturbs the macrophage inflammatory response and hampers the control of the bacterial load, we are currently characterizing with more detail the role of CD43 in shaping the inflammatory response, and pulmonary pathology of CD43-deficient mice challenged with M. tuberculosis. Based on these data, the relevance of the specific interaction between CD43 and Cpn60.2 or DnaK in the regulation of the immune response against M. tuberculosis will be discussed.

Promising anti-inflammatory and antimicrobial peptide isolated from the mexican tree frog Pachymedusa dacnicolor in the treatment of inflammatory skin diseases

Elizabeth Fajardo Brigido[1,2], Veronica Martinez[1,2], Estefanía Alemán[1,2], Thierry Drujon[3,4], Christophe Piesse[4,5], Sandra Rodríguez-Martínez[6], Christophe Combadiere[7,8], Claire Lacombe[3,4,9], Yvonne Rosenstein[1,2], Constance Auvynet[1,2]

[1]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
[3]École Normale Supérieure, Paris, France
[4]Sorbonne Universités, Paris, France
[5]FR3631 Institut de biologie Paris Seine, Paris, France
[6]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico
[7]ERL8255 Centre d'Immunologie et de Maladies Infectieuses (CIMI), Paris, France
[8]Université Pierre et Marie Curie, Paris, France
[9]Faculté des Sciences et Technologie, Université Paris-Est Créteil Val de Marne, Créteil, France
lfajardo@lcg.unam.mx; vmartinezo@unal.edu.co; steph@ibt.unam.mx; thierry.drujon@upmc.fr; christophe.piesse@upmc.fr; sandrarodm@yahoo.com.mx; christophe.combadiere@upmc.fr; claire.lacombe@upmc.fr; yvonne@ibt.unam.mx; constance@ibt.unam.mx

Keywords: Anti-Inflammatory Agents, Skin Diseases, antimicrobial peptides, Inflammation resolution, Frog peptides

Inflammatory and antimicrobial diseases are a major burden for the society today and fighting against them is a national and WHO strategic priority. Nowadays, most of the treatments available on the market to fight inflammatory diseases are anti-inflammatory drugs, such as corticosteroids or immunomodulators (IFN beta) that lack cellular specificity and lead to numerous side effects. Moreover, besides suppressing undesired inflammation and reducing disease progression, they also diminish protective functions of the immune system. In addition, treating infectious diseases is more and more challenging, due to the increase of microbial resistance to antimicrobial drugs.

Specifically controlling the inflammatory process without compromising the ability of the body to combat infections is an essential feature of anti-inflammatory therapy.

In this study, we characterized a new peptide isolated from the mexican tree frog, Pachymedusa dacnicolor, that exhibits both, anti-inflammatory and antimicrobial properties. Indeed, in vitro, this peptide specifically kills immune cells without

compromising the integrity of non-immune cells and kills Gram-positive and Gram-negative bacteria. In vivo, this peptide reduces inflammation and disease progression in the preclinical murine model of psoriasis and dermatitis. Thus, our peptide could be a promising drug in the treatment of inflammatory skin diseases.

Molecular and genetic description of primary immunodeficiencies in mexican pediatric patients

Maria Elena Bravo-Adame[1], Beatriz Adriana Llamas-Guillen[2], Nina Pastor[1], Gabriela Lopez-Herrera[3], Maria Edith Gonzalez-Serrano[3], Lucero Valenzuela-Vazquez[1], Paul Gaytan[4], Jorge Yañez[4], Ivan Martinez-Duncker[1], Andre Veillette[5], Sara Elva Espinosa-Padilla[3], Maria Enriqueta Nuñez[6], Ana Paola Macias-Robles[7], Sergio Rosenzweig[8], Julie E Niemela[8], Luigi Daniele Notarangelo[8], Mario Ernesto Cruz-Muñoz[1]

[1]Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico
[2]Hospital del Niño y el Adolescente Morelense, Emiliano Zapata, Mexico
[3]Instituto Nacional de Pediatria, Mexico City, Mexico
[4]Instituto de Biotecnologia UNAM, Cuernavaca, Mexico
[5]Institute Of Clinical Research De Montreal (IRCM), Montréal, Canada
[6]Hospital Civil de Guadalajara, Guadalajara, Mexico
[7]Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Mexico
[8]National Institutes of Health (NIH), Bethesda, United States
mali_bravo@yahoo.com; beadriluz@hotmail.com; nina@uaem.mx; lohegabyqbp@gmail.com; megpetite@gmail.com; luvav@outlook.com; paul@ibt.unam.mx; jay@ibt.unam.mx; duncker@uaem.mx; andre.veillette@ircm.qc.ca; saraelvaespino@gmail.com; nunezen@hotmail.com; paola_maro@hotmail.com; sergio.rosenzweig@nih.gov; jniemela@cc.nih.gov; luigi.notarangelo2@nih.gov; mario.cruz@uaem.mx

Keywords: SCID, Primary immunodeficiency (PID), ZAP70 deficiency, IL2RG mutation, RAG2 mutation

Introduction: Severe combined immunodeficiency (SCID) is one of the most severe forms of primary immunodeficiency diseases, and is characterized by low, absent, or non-functional T lymphocytes. This condition is fatal without early and definitive therapy. In fact, outcomes may be improved if diagnosis and treatment occur within the first months of life. Although several approaches are currently used to diagnose SCID patients, different assays must be used in order to properly evaluate the existence of all forms of SCID. Nowadays, next generation sequencing strategies such as whole exome sequencing allows for rapid and accurate diagnosis of specific causes of SCID. Combined with initial molecular characterization of patients' peripheral blood mononuclear cells, this strategy overcomes the limitations of other methodologies during SCID screening (TREC, for example). Whole exome sequencing would allow the detection of T cell lymphopenias due to defects downstream of the T cell receptor rearrangement, such as Zap70 deficiency for example, where impairment of T cell function, despite a quantitatively normal T cell number, would not be appropriately diagnosed using the TREC assay only. In addition to the advantages of next generation

sequencing techniques for the diagnosis of Mendelian inherited diseases, primary immunodeficiencies provide a unique human model in natura to study the mechanisms of action of proteins that regulate immune function. By creating in vivo and in vitro models to reproduce specific mutations, we can unravel the role of a single protein in the development of the disease. In this study, we present the diagnosis strategy for the identification of the genetic causes of disease in five SCID patients, as well as the importance of reproducing the genetic defects in vitro using cell line models that will allow us to further understand the biology of these diseases.

Methods: Initially, molecular characterization of lymphocytic populations was assessed through flow cytometry, followed by functional evaluation of T cells. The search for mutations in patient samples was performed through whole exome sequencing and results were confirmed by Sanger sequencing. Protein mutants were then submitted to structural analysis and modeling. In some cases, mutations were introduced into human cell lines in order to depict the functional defects in T cell signaling that occurred as a consequence of the identified mutations, and their association with the patient's clinical manifestations.

Results: Genetic analyses allowed us to find the mutations responsible for the patients' phenotypes. We report here two SCID patients carrying mutations in the RAG2 gene (c.464 T>C, p.L155P and c.685 C>T, p.R229W in the first patient, and c.104 G>T, p.G35V in the second patient). Zap70 defects were found in two patients, either at the level of protein expression or function. In one patient, two novel mutations in the ZAP70 gene were described (c.37 G>C, p.G13R and c.1198 C>T, p.R400W). Both mutations caused partial absence of the Zap70 protein and their structural consequences were predicted by protein 3D modeling. Moreover, we investigated the impact of these mutations using the Zap70 deficient Jurkat-derived T cell line P116, expressing ZAP-70 WT or ZAP-70 mutants through retroviral infections. We observed that both mutations were ineffective to promote TCR-dependent signals. Interestingly, the second patient showed normal Zap70 expression, but downstream signaling events were impaired, suggesting functional alterations of this kinase. Finally, a novel mutation in the IL2RG gene was identified in a X-SCID patient (c.667 G>T, p.V223F), suggesting that this alteration is the cause of the disease.

Conclusions: In Mexico, next generation technologies are beginning to be applied for primary immunodeficiencies research. However, it remains necessary to promote the use of these and other complementary techniques in order to improve research, diagnosis, and treatment. We suggest that following the identification of mutations, the

use of molecular biology techniques that will allow the generation of in vitro models of human primary immunodeficiencies will represent a major advantage to further understand these diseases. By providing information about the protein niche in natura, this strategy represents a potential molecular and genetic approach for the identification and study of primary immunodeficiencies that would ultimately contribute to patient diagnosis, treatment, and welfare.

Molecular mechanisms of ubiquitin-mediated NETosis regulation in patients with systemic lupus erythematosus

Diana Gómez-Martín[1], Ana Barrera-Vargas[1], Carmelo Carmona-Rivera[2], Javier Merayo-Chalico[1], Jiram Torres-Ruiz[1], Jorge Alcocer-Varela[1], Mariana Julieta Kaplan[2]

[1]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
[2]National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, United States
diana.gomezm@incmnsz.mx; anabar9@hotmail.com; carmelo.carmona-rivera@nih.gov; oyarem@hotmail.com; josetorresruiz85@gmail.com; jorgealcocer2@gmail.com; mariana.kaplan@nih.gov

Keywords: Cytokines, Macrophages, Ubiquitin, systemic lupus erythematosus (SLE), NETs

Rationale: Neutrophil extracellular traps (NETs) have recently been associated with the pathogenesis of systemic lupus erythematosus (SLE)(Lood et al., 2016). Different post translational modifications (PTM) have been described in NET components(Liu et al., 2012). Ubiquitination is a PTM traditionally associated with protein degradation; however, abnormalities in this process have also been recently associated with peripheral tolerance defects in SLE patients(Gomez-Martin et al., 2013). Also, extracellular ubiquitin and its signaling through CXCR4 have been implicated in immune system regulation(Saini et al., 2010). However, whether ubiquitinated proteins are externalized during NETosis and modify cellular responses in target cells remains unclear.

Aim: To assess if ubiquitinated proteins potentially present in neutrophil extracellular traps (NETs) can modify cellular responses and induce inflammatory mechanisms in systemic lupus erythematosus (SLE) patients and healthy controls.

Materials and Methods: We studied 70 subjects with SLE that fulfill the ACR or SLICC criteria, who were either in remission (SLEDAI = 0 points) or had active disease (SLEDAI ≥ 6 points), and 70 age and gender-matched healthy controls. Neutrophils were isolated by density centrifugation through Ficoll-Hypaque, and then through dextran as previously described(Carmona-Rivera et al., 2015). Erythrocyte lysis was performed with hypotonic saline solution (0.2%). Low density granulocytes (LDGs) were isolated from lupus peripheral blood mononuclear cells by magnetic positive selection. In neutrophils derived from healthy subjects, NETs were induced by LPS stimulation. In SLE patients, NETs were either induced with LPS stimulation or they

were formed without additional stimuli (spontaneous). We quantified ubiquitin content in NETs by Western Blot, ELISA, immunofluorescence and confocal microscopy. Ubiquitination of NET proteins was assessed by immunoprecipitation and Western Blot. Anti-ubiquitinated MPO antibodies were detected by ELISA. Monocyte-derived macrophages from both patients and controls were isolated and stimulated with NETs or ubiquitin in the presence or absence of a CXCR4 inhibitor or chloroquine. Calcium flux and cytokine synthesis were measured following these stimuli by fluorescence spectrometry and ELISA, respectively.

Results: NETs contain ubiquitinated proteins, with a lower expression of polyubiquitinated proteins in SLE subjects than in healthy subjects. A differential ubiquitination pattern was found between SLE subjects and healthy controls by Western Blot analysis, with a lower expression of K63 polyubiquitinated proteins in SLE subjects ($p<0.05$). Myeloperoxidase (MPO) is present in ubiquitinated form in NETs. SLE patients develop anti-ubiquitinated MPO antibodies. When compared to controls, SLE patients displayed significantly increased titers of anti-Ub-MPO (0.165 vs 0.094 O.D., $p<0.01$) as well as anti-native MPO antibodies (0.144 vs 0.076 O.D., $p<0.01$). Furthermore, when comparing antibodies against native MPO and the Ub-MPO proteins, there was a higher concentration against the latter in SLE subjects (0.144 vs 0.165 O.D., $p<0.01$). We also found that anti-Ub MPO titers positively correlate with SLEDAI score ($r= 0.44$, $p<0.01$), and negatively correlate with complement components (C3: $r= -0.428$, $p<0.01$; C4: $r=-0.432$, $p<0.01$). Furthermore, stimulation of monocyte-derived macrophages with NETs from control or SLE NDGs or from SLE LDGs, increased calcium flux was also triggered, and it similarly decreased in the presence of a CXCR4 inhibitor. These results indicate that there is activation of CXCR4 by ubiquitin in NETs. When the response from SLE macrophages was evaluated, the largest increase in calcium flux was observed with control NETs, while the lowest increase occurred with LDG NETs. These findings correlated with the ubiquitin concentration gradient found by ELISA in NETs. In addition, stimulation with NETs led to an enhanced cytokine (TNF-α, IL-6 and IL-10, $p<0.05$) production in macrophages from SLE patients when compared to controls, which was hampered (TNF-α, IL-6, $p<0.01$ and <0.05, respectively) by inhibition of NET internalization by chloroquine treatment.

Conclusion: In summary, this is the first study to find the presence of polyubiquitinated proteins in NETs, with a differential profile between SLE patients and healthy controls. NETs from SLE subjects displayed a deficient K63 mediated ubiquitination, which could be related to its role in oxidative responses. MPO was found to be ubiquitinated in NETs and our data suggest it is a target of humoral responses in SLE. Furthermore, ubiquitin present in NETs promotes an increase in calcium flux in macrophages through CXCR4

signaling. Lupus macrophages synthesize inflammatory cytokines in response to NET internalization, a process that could be partly mediated by TLR4 and 9(Macfarlane and Manzel, 1998). Overall, abnormalities in mechanisms involved in NET internalization and the extracellular ubiquitination pathway could play important roles in the development of inflammatory responses in SLE.

REFERENCES

Carmona-Rivera, C., Zhao, W., Yalavarthi, S., and Kaplan, M.J. (2015). Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. Ann Rheum Dis 74, 1417-1424.

Gomez-Martin, D., Ibarra-Sanchez, M., Romo-Tena, J., Cruz-Ruiz, J., Esparza-Lopez, J., Galindo-Campos, M., Diaz-Zamudio, M., and Alcocer-Varela, J. (2013). Casitas B lineage lymphoma b is a key regulator of peripheral tolerance in systemic lupus erythematosus. Arthritis Rheum 65, 1032-1042.

Liu, C.L., Tangsombatvisit, S., Rosenberg, J.M., Mandelbaum, G., Gillespie, E.C., Gozani, O.P., Alizadeh, A.A., and Utz, P.J. (2012). Specific post-translational histone modifications of neutrophil extracellular traps as immunogens and potential targets of lupus autoantibodies. Arthritis Res Ther 14, R25.

Lood, C., Blanco, L.P., Purmalek, M.M., Carmona-Rivera, C., De Ravin, S.S., Smith, C.K., Malech, H.L., Ledbetter, J.A., Elkon, K.B., and Kaplan, M.J. (2016). Neutrophil extracellular traps enriched in oxidized mitochondrial DNA are interferogenic and contribute to lupus-like disease. Nat Med 22, 146-153.

Macfarlane, D.E., and Manzel, L. (1998). Antagonism of immunostimulatory CpG-oligodeoxynucleotides by quinacrine, chloroquine, and structurally related compounds. J Immunol 160, 1122-1131.

Saini, V., Marchese, A., and Majetschak, M. (2010). CXC chemokine receptor 4 is a cell surface receptor for extracellular ubiquitin. J Biol Chem 285, 15566-15576.

SEB stimulation induces functional pathogenic features in Th17 cells from psoriasis patients

Octavio Castro-Escamilla[1,2], Cristina Aguilar-Flores[2,3], Luz Maria Mora-Velandia[2], Gibrán Pérez-Montesinos[4], Laura Bonifaz[2,5]

[1]Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[2]Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico
[3]Instituto Politécnico Nacional, Mexico City, Mexico
[4]Centro Dermatológico Dr. Ladislao de la Pascua, Ciudad de Mexico, Mexico
[5]FOCIS Centre of Excellence, Ciudad de México, Mexico
larenmc@msn.com; acristins@outlook.com; luzmv27@gmail.com; branpm1@gmail.com; labonifaz@yahoo.com

Keywords: Psoriasis, SEB, Pathogenic, Th 17 cell, IL-17-IFN-γ

Background: Psoriasis is an inflammatory skin disease that affects between 2-3% worldwide population without gender preference. Psoriatic lesions are well-delimited plaques characterized by erythema, induration and scales, where the evidence suggests that immune system has a key role in the physiopathology. It has been demonstrated that cytokines produced by Th17 lymphocytes such as IL-17 and IL-22 are essential to maintain the inflammatory conditions in skin. However, it has been proposed that there are two types of Th17 cells. Conventional Th17 cells are characterized by expression of IL-17 and RORγt, while pathogenic Th17 cells express IFN-γ, RORγt and T-bet. There are several psoriasis-triggering factors such as stress, traumatism and bacterial infections. Recently, an analysis of lesional skin microbiome in psoriasis patients reveals an increased presence of Staphylococcus aureus and its superantigens such as Enterotoxin-B (SEB) which has been associated with psoriasis severity. However, the phenotype and function of Th17 cells in the presence of SEB remains unknown in psoriasis patients.

Aim: To evaluate the conventional and pathogenic Th17 transcriptional phenotypes and their functional properties in skin biopsies from psoriasis patients after SEB stimulation.

Material and Methods: In this study we included fifty psoriasis patients and obtained biopsies from lesional and non-lesional skin and peripheral blood. We analyzed the Th17 cells phenotype and cytokine production through flow cytometry and confocal microscopy.

Results: We found an increased frequency of Th17 cells in lesional skin. In situ analyzes show the presence of CD4+ RORγt+ cells and CD4+ IL-17+cells. We identified both Th17 cell phenotypes according to RORγt, Runx1 and T-bet expression in skin biopsies. Remarkably, after SEB stimulation, conventional Th17 cells were decreased and pathogenic Th17 cells were increased in lesional skin. In addition, the expression of RORγt, Runx1 and T-bet were increased in Th17 cells after SEB treatment. In situ, we found some CD4+ lymphocytes that simultaneously express IL-17 and IFN-γ in lesional skin. After SEB activation, the pathogenic phenotype was intensified in Th17 cells due to the increased expression of T-bet, acquiring functional features such as IFN-γ production and a reduction of IL-17 expression. We found that Th17 cells from lesional skin have an increased IFN-γ production and proliferation capability compared to Th17 cells from blood. SEB treatment promotes a pathogenic environment revealed by low quantities of IL-17 and IL-9, but high levels of IFN-γ.

Conclusion: The Th17 pathogenic phenotype and functional features are intensified by SEB stimulation and these findings may be relevant in maintaining the local inflammatory process in psoriasis. Therefore, new therapies focused in blocking the plasticity or the acquisition of pathogenic features by Th17 cells could also be promising in psoriasis treatment.

A prospective study unveils innate and adaptive immunity abnormalities as key risk factors for infections in patients with systemic lupus erythematosus: Results from the Germen cohort

José Jiram Torres-Ruiz[1], Ricardo Vázquez-Rodríguez[1], Sandra Rubí Morales-Padilla[1], Roberto Adrián Reyna-de-la-Garza[1], Guillermo Juárez-Vega[2], Francisco Javier Merayo-Chalico[1], Nancy Raquel Mejía-Domínguez[2], Jorge Carlos Alcocer-Varela[1], Diana Gómez-Martín[1]

[1]*Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico*
[2]*Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico*
josetorresruiz85@gmail.com; richard.1350@hotmail.com; san.mrls@hotmail.com; roberto.reynadlg@gmail.com; guillermovega@cic.unam.mx; oyarem@hotmail.com; nancy.mejia@gmail.com; jorgealcocer2@gmail.com; d_gomar@hotmail.com

Keywords: Infections, SLE, Th17, B-cells, TLR-2, LDG

Introduction: Infections are one of the three main causes of hospitalization and death in patients with systemic lupus erythematosus (SLE) at any time throughout the disease course. Based on retrospective studies, the use of glucocorticoids and immunosuppressive therapy, damage accrual, renal involvement, smoking [1], hypocomplementemia and lymphopenia [2, 3] have been acknowledged as risk factors for the development of infection in patients with SLE. Nevertheless, 25% of infections in patients with SLE occur at diagnosis in the absence of immunosuppressive therapy [4] and therefore, the immunological abnormalities in SLE may play a key role in the unique infection predisposition in these patients. SLE is a complex disease which is known to have altered T cell homeostasis, with higher levels of Th1, Th2 and Th17 cells and lower percentage of regulatory T cells [5]. Furthermore, monocytes of patients with SLE show phagocytosis defects [6] and it has been shown in animal models that the recognition of bacterial amyloid-DNA composites by monocytes expressing TLR-2 promotes an interferonogenic response, which might induce disease activity [7]. Neutrophils from patients with SLE are more prone to die through a special mechanism of cell death called NETosis and a neutrophil subset called low density granulocytes are a major source of neutrophil extracellular traps (NETs) and type I IFN [8]. NETs contain antigenic chromatin and antimicrobial peptides such as LL-37 [8] and it is unknown if the expression of this molecule is deficient among patients with SLE that develop infections. The aim of this study was to assess if the innate and adaptive immunological abnormalities of patients with SLE represent a risk factor for the development of infections.

Methods: A prospective cohort study of 59 SLE patients according to the ACR SLICC criteria was undertaken. At baseline and subsequently every 3 months, patients were clinically assessed to register the immunosuppressive therapy and disease activity with the SELENA/SLEDAI and BILAG scores. We performed immuno-typification by multiparametric flow cytometry of CD4+ T lymphocytes subsets, B cells, NK cells, low density granulocytes (LDG), as well as monocytes subtypes and their expression of TLR2. In addition, the production of neutrophil extracellular traps (NETs) and their LL-37 expression were evaluated by fluorescence spectrometry and confocal microscopy. These parameters were also assessed at the time of the primary outcome, which was a clinically diagnosed infection.

Results: Twenty-one patients (35%) developed an infection during 12 months of follow-up. Infections occurred mainly in the upper (16%) and lower (29%) respiratory tract, and 25% developed herpes zoster. At baseline, the expression of TLR2 in total monocytes was reduced by 56% in patients with SLE in comparison to healthy subjects (mean fluorescence intensity (MFI): 9567 (5552-13848) vs 16890 (11124-22256), p=0.005). During the initial evaluation, patients who developed infections had higher anti-dsDNA titers (130 (79-793) vs 78.45 (27.1-223), p = 0.039), higher percentage of Th17 cells (1.8 vs 0.9, p = 0.01) and absolute numbers of B cells/mm3 (88 vs 253, p = 0.028), Th17/mm3 (11 vs 3, p = 0.007) and LDG/mm3 (17.7 vs 8, p = 0.006). To encompass the variability of data over time, we calculated the delta (Δ) value of every parameter in 2 consecutive visits and performed a logistic regression (R2 =0.77). The model was adjusted for the delta of immunosuppressive therapy, disease activity scores and laboratory parameters. The RR (95% CI) for infection were: ΔHemoglobin (8.3 (2.3-89.7, P<0.05), -ΔTLR2 in monocytes (0.999 (0.9995-0.9999, P<0.05), -Δtotal monocytes (0.991 (0.9813-0.9988, P<0.05) and -ΔB cells (0.993 (0.9871-0.9993, P<0.05). During the infectious event, SLE patients had higher levels of LDG (10% (4.8-19.6) vs 6.49% (2.2-18.8), P=0.016) and a higher expression of LL-37 in LPS-induced NETs (MFI: 134 (72.5-297) vs101 (58.2-160.75), P=0.013).

Conclusions: Patients who developed infection had baseline B cell lymphopenia and higher levels of anti-dsDNA antibodies, LDG and Th17. LDG are a source of type I IFN, which is known to promote the Th17 differentiation [9]. Type I IFN produce a B cell hyperactivation that may account for the higher levels of anti-dsDNA antibodies and lately it promotes activation-induced necroptosis in B-cells [10]. This mechanism may lead to B-cell lymphopenia and to a reduced the B-cell repertoire in patients with SLE, augmenting the risk of infection. During follow-up, the decrease in hemoglobin, B cells, monocytes as well as their TLR-2 expression were independent risk factors for infections. TLR-2 has many ligands, and human polymorphisms of this receptor are

a risk factor for viral, bacterial, fungal and mycobacterial infections [11]. Among the factors that may promote a lower expression of TLR-2, steroids have shown to diminish the expression of TLR-2 in airway epithelial cells [12] but further studies are needed to assess if this mechanism also apply to patients with SLE. Finally, our findings suggest that immunologic parameters characteristic of high SLE activity are independent risk factors for infections and therefore, it is important to treat the patients in a timely manner and to achieve remission as soon as possible.

REFERENCES

1. Rua-Figueroa I., Lopez-Longo J., Galindo-Izquierdo M., et al. Incidence, associated factors and clinical impact of severe infections in a large, multicentric cohort of patients with systemic lupus erythematosus. Semin Arthritis Rheum 2017;47(1):38-45.
2. Barrera-Vargas A., Gomez-Martin D., Merayo-Chalico J., Ponce-De-Leon A., Alcocer-Varela J. Risk factors for drug-resistant bloodstream infections in patients with systemic lupus erythematosus. J Rheumatol 2014;41(7):1311-6.
3. Merayo-Chalico J., Gomez-Martin D., Pineirua-Menendez A., Santana-De Anda K., Alcocer-Varela J. Lymphopenia as risk factor for development of severe infections in patients with systemic lupus erythematosus: a case-control study. QJM 2013;106(5):451-7.
4. Ng W. L., Chu C. M., Wu A. K., Cheng V. C., Yuen K. Y. Lymphopenia at presentation is associated with increased risk of infections in patients with systemic lupus erythematosus. QJM 2006;99(1):37-47.
5. Rother N van der Vlag J. Disturbed T Cell Signaling and Altered Th17 and Regulatory T Cell Subsets in the Pathogenesis of Systemic Lupus Erythematosus. Front Immunol 2015;6:610.
6. Katsiari Cg Liossis SN, Sfikakis PP. The pathophysiologic role of monocytes and macrophages in systemic lupus erythematosus: a reappraisal. Semin Arthritis Rheum 2010;39(6):491-503.
7. Gallo P. M., Rapsinski G. J., Wilson R. P., et al. Amyloid-DNA Composites of Bacterial Biofilms Stimulate Autoimmunity. Immunity 2015;42(6):1171-84.
8. Garcia-Romo Gs Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, Punaro M, Baisch J, Guiducci C, Coffman RL, Barrat FJ, Banchereau J, Pascual V. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med 2011;3(73):73ra20. doi: 10.1126/scitranslmed.3001201.
9. Biswas Ps Aggarwal R, Levesque MC, Maers K, Ramani K. Type I interferon and T helper 17 cells co-exist and co-regulate disease pathogenesis in lupus patients. Int J Rheum Dis 2015;18(6):646-53.
10. Fan H., Liu F., Dong G., et al. Activation-induced necroptosis contributes to B-cell lymphopenia in active systemic lupus erythematosus. Cell Death Dis 2014;5:e1416.
11. Noreen M., Arshad M. Association of TLR1, TLR2, TLR4, TLR6, and TIRAP polymorphisms with disease susceptibility. Immunol Res 2015;62(2):234-52.
12. Winder A. A., Wohlford-Lenane C., Scheetz T. E., et al. Differential effects of cytokines and corticosteroids on toll-like receptor 2 expression and activity in human airway epithelia. Respir Res 2009;10:96.

Bone marrow organoid-like reveals functional hierarchy governed by the niche in B-cell leukemia-initiating cells

Juan Carlos Balandrán[1,2,3], Gustavo Iñaki López Reyes[1,4], Jiovanni Amador Cardoso[1,4], Gabriela Zamora Herrera[1,4], David Salomón Dozal[5], Antonio Sandoval Cabrera[5], Vadim Pérez Koldenkova[6], Blanca Félix Bañuelos García[1], Henry Quintela Nuñez del Prado[6], Elva Jiménez Hernández[7], Lilia Adela García Stivalet[1], José Alejandro Limón Flores[1], Vianney Ortiz Navarrete[2,3], Monica L Guzman[8], Rosana Pelayo[1,3]

[1]Instituto Mexicano del Seguro Social (IMSS) Delegación Puebla, Metepec-Atlixco, Mexico
[2]Centro de Investigación y de Estudios Avanzados (CINVESTAV) - IPN, Ciudad de México, Mexico
[3]Federation of Clinical Immunology Societies (FOCIS), Mexico City, Mexico
[4]Benemérita Universidad Autónoma de Puebla, Puebla, Mexico
[5]Instituto Materno Infantil del Estado de México, Toluca, Mexico
[6]Instituto Mexicano del Seguro Social (IMSS), Ciudad de México, Mexico
[7]Secretaría de Salud, Ciudad de México, Mexico
[8]Weill Cornell Medicine, New York, United States
jcbalandran@gmail.com; ponnylr@gmail.com; jiovanni-amador@hotmail.com; gabriela.zamorah@alumno.buap.mx; david_dozal@hotmail.com; sandoval.mx@gmail.com; vad.perez@gmail.com; pipucho_sol@hotmail.com; drhenryquintela@yahoo.com; elvajimenez@yahoo.com; lilia.garcia@imss.gob.mx; jose.limonf@imss.gob.mx; vortiz@cinvestav.mx; mlg2007@med.cornell.edu; rosanapelayo@gmail.com

Keywords: Mesenchymal Stromal Cells, Tumor Microenvironment, Leukemia-initiating cells, B-cell leukemia, organoid-like, hypoxic niches

Introduction: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer around the world where the highest rates and poorest prognosis have been reported for pediatrics of Hispanic ethnicity. ALL is heterogeneous and characterized by exacerbated oligoclonal proliferation of lymphoid precursors in bone marrow (BM) at the expense of normal hematopoiesis. A comprehensive understanding of the biological factors that influence development and pathobiology of leukemia-initiating cells (LIC) is still missing as immunophenotype cannot distinguish LIC from normal stem/progenitor cells. Furthermore, serial transplantation in xenograft models suggests the lack of hierarchal organization in lymphoid leukemia origins. LIC have been implicated in the pathogenesis of relapse by evading chemotherapy attributed to their quiescence and intercommunication within the BM niche. Elucidating the interactions of LICs with their microenvironment is critical for understanding ALL origins, response and resistance to therapy. BM is a three-dimensional tissue tightly regulated, composed by osteoblasts, endothelial cells, mesenchymal cells and innervation of CNS which

jointly supports hematopoiesis along life. Studies in mice have revealed that a special population of mesenchymal stromal cells (MSC), the Nestin+LeptinR+PDGFR+ CXCL12-abundant reticular cells, conform niches to support hematopoietic stem cells (HSC) and early lymphoid precursors through CXCL12, SCF and IL-7. We previously reported that human mesenchymal component from ALL BM differ from NBM in some niche properties resulting in a biological advantage during leukemic development benefited from increased production of pro-inflammatory cytokines and the concomitant abnormal loss of CXCL12 and SCF (1). In this work, we have developed a 3D BM organoid-like model that better mimics mesenchymal hypoxic niches capable of maintain LIC properties and their inter/dependency of the niche.

Methods: Primary human MSC (hMSC) from ALL BM at clinical debut and NBM from healthy individuals were isolated from BM aspirates by their plastic-adherent properties and further characterized by classic immunophenotype and differentiation (osteogenic, chondrogenic and adipogenic) potentials. MSC were induced to form stromal spheroids (3D) in non-adherent conditions and changes in size, phenotype and proliferation capability were recorded. Molecules involved in niche-interaction roles were measured in 3D and 2D supernatants. BM CD34+ cells from ALL patients were co-cultured with 3D spheroids or monolayers in lymphoid conditions (hrFlt3-L, hrSCF, hrIL-7). As leukemic cells migrate and colonize spheroids, cells were harvested from inside (3D-in) and outside (3D-out) of spheroids to evaluate their proliferation, phenotype, and stem cell properties including in vivo xenotransplantation assays. Organoid-like structures were treated with chemotherapeutic drugs used in the standard care and viability on ALL cells were determined.

Results and Discussion: Primary hMSC from healthy controls and ALL patients form functional spheroids with no evident changes in size or phenotype but 3D structures promote secretion of CXCL12, SCF, IL-7, IL-15 and IL-10 compared to monolayers (2D). Their biological impact on primary ALL cells, was tested in co-cultures using ALL- and NBM-stromal spheroids, monolayer (2D) and stromal-free (SF) systems. Viability and frequencies of ALL CD34+ cells were significantly higher in the 3D structures after one week of culture. In addition, lymphoid (B, NK and dendritic linages) differentiation potential in long term co-cultures of normal CD34+ cells was promoted by 3D system. Importantly, we tested the stem cell function using in vivo reconstitution in NSG mice from NBM and ALL CD34+ cells harvested from 3D co-cultures. We found an increase in engraftment ability in 3D compared with 2D and SF scenarios, suggesting an enrichment for LICs in the 3D system. Of note, a conspicuous population (0.3 - 4% of MNC) was able to colonize spheroids, and to determine the mechanism behind this organoid-colonization we block CXCR4/CXCL12 by using a

CXCR4 inhibitor and found their partial migration. Interestingly, the most primitive (CD34+) were inside the 3D system and preferentially in a quiescent (G0) status, while cells surrounding organoid-like structures (3D-out) were CD34- and proliferating. In addition, we found that side population is also significantly higher in the 3D-in compartment. Because hypoxia is a biophysical property of BM, we evaluated hypoxia using pimonidazol (pimo) a drug that bind proteins in hypoxic state widely used to indirectly measure low O2 concentrations. Anti-pimo staining by fluorescent microscopy confirmed hypoxic zones within the organoid-like structures in MSC as well as in CD45+ cells suggesting that ALL-organoid-resident cells colonize hypoxic zones. Taken these findings together, putative LIC are selectively enriched in hypoxic milieu within the organoid-like system, which was confirmed by limiting dilution assays. Briefly, same numbers of ALL cells harvested from 3D-in, 3D-out, 2D or SF ALL were transplanted into NGS mice. Animals were monitored weekly for leukemic engraftment by assessing hCD45+ cells in peripheral blood (PB). As expected, the more leukemic cells were injected, the faster development of leukemia consisting with increasing frequency of LICs, specially in the 3D-in cohorts, confirming the LIC frequency-enrichment by the niche. When conducting serial spheroid plating, organoid-like resident cells (3D-in) were capable of re-colonizing secondary systems more efficiently than ALL cells from 3D-out, indicating a functional hierarchy in ALL regulated by the niche, where only a small population of LIC cells is able to colonize stromal organoids and recapitulate leukemia in vivo.

Due to the increasing evidence showing the ability of leukemic cells to hide within the niche during chemotherapy, we used our novel ALL-organoid-like system to investigate chemoresistance, finding that 3Din ALL cells were resistant and that this property is favorably maintained when the 3D-in cells were cultured outside the organoids and exposed to same drugs, suggesting that chemoresistance is a behavior promoted by the niche. Furthermore, ALL-organoids were isolated upon chemotherapy treatment and tested for lymphoblasts reconstitution from resistant clones, strongly suggesting that relapse or MRD cells may reemerge from protective niches where functional LIC are selected.

Conclusion: A functional cellular hierarchy in the initiation of ALL, governed by the mesenchymal niche Nestin+CXCL12+SCF+IL7+ is demonstrated by providing the microenvironmental structure of leukemic BM. Our 3D organoid/like novel approach would be relevant to the understanding of the pathobiology of the disease.

REFERENCE

(1) Balandrán JC, Purizaca J, Enciso J, Dozal D, Sandoval A, Jiménez-Hernández E et al. Pro-inflammatory-Related Loss of CXCL12 Niche Promotes Acute Lymphoblastic Leukemic Progression at the Expense of Normal Lymphopoiesis. Front. Immunol. (2017) 7:666. doi: 10.3389/fimmu.2016.00666

IL1β negatively regulates antiviral immunity in a mouse model of viral hepatitis

Maite Duhalde Vega[1,2], Mathias Jeldres[3], Maria Cristina Cuturi[4], Marcelo Hill[3,5]

[1]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[2]Institut Pasteur de Montevideo, Montevideo, Uruguay
[3]Centre for Translational Immunology. Institut Pasteur de Montevideo. FOCIS Centre of Excellence, Montevideo, Uruguay
[4]INSERM UMR1064 Centre de Recherche en Transplantation et Immunologie, Nantes, France
[5]Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
maiduve@gmail.com; mjeldres@gmail.com; Maria-Cristina.Cuturi@univ-nantes.fr; mhill@pasteur.edu.uy

Keywords: MHV, PD-1, IL1b, TMEM176, NLRP3 inflammasome

Introduction: Virus triggered innate and adaptive immune responses in the host to control infection, but this mechanism can also damage the host. Thus, antiviral immunity is a double-edged sword between immunoprotection and immunopathology, that it is constantly regulated to avoid unnecessary damage. The production of proinflammatory cytokines is a critical step for an effective innate response and is also a pivotal mechanism by which the innate immune system influences the development of adaptive immune responses. The inflammasomes play a crucial role in the immune response to viral infection. They are multiproteins complexes that induce downstream immune responses after the encounter with pathogens or cell damage signals. Activation of NLRP3 inflammasome triggers the cleavage of caspase 1 and maturation of IL1β, critical steps for an efficient infection control.

The positive relation between NLRP3 activity, IL1β release and adaptive immunity has been exhaustively described on several models, but hepatitis seems to be negatively influenced by the inflammasome. In HCV patients, IL1β level is augmented in plasma and liver and, this value, it has been strongly associated with liver immunopathology and elevated viral load. Moreover, expression of Tmem176b, an intracellular cation channel has been associated to HCV clearance. Recently, we have identified Tmem176b as a novel modulator of the NLRP3 inflammasome activity. Thus, we speculated that Tmem176b might promote viral clearance by inhibiting inflammasome activation.

Therefore, the aim of this work was to further analyze the role of the NLRP3-IL1β axis in the initiation of adaptive immune responses in a model of viral hepatitis to identify novel regulatory mechanisms that allow modulating antiviral immunity. We investigate the role of Tmem76b as a NLRP3 activity modulator in this model.

Materials and Methods: Cells and animals were infected with Mouse Hepatitis Virus strain A59 (MHV), a coronavirus which induces hepatitis and activates NLRP3 inflammasome-dependent IL1β release. We use WT and Tmem176b-deficient mice. Tmem176b deficient mice have an excessive release of IL1β after NLRP3 activation. We performed in vitro and in vivo inflammasome activation experiments. We developed survival analysis, WB, ELISA and flow cytrometry assays to determine immunological, biochemical and survival parameters of the infected mice.

Results: To determined the role of Tmem176b on MHV-induced NLRP3 activation. BMDCs derived from Tmem176b-deficient mice and wt mice were infected with MHV and inflammasome activation was analyzed. Data have shown that Tmem176b-deficient mice release more IL1β than wt mice ($p \leq 0.001$) and Caspase 1activity was exacerbates on Tmem176b-deficient mice ($p \leq 0.001$). In addition, MHV-induced IL1β release rely on NLRP3 activation, since infected BMDCs derived from caspase 1-deficient mice or NLRP3-deficient mice, do not release IL1β after infection. This data revealed that Tmem176b is a modulator of NLRP3 activity after MHV infection.

Afterward, in vivo studies have shown that the lack of Tmem176b aggravates MHV-induced hepatitis. Survival rates analyses revealed that Tmem176b-deficient mice were highly susceptible to MHV infection, with 100% of animals dead at 6.5 days post-infection (dpi), while 75% of Tmem176b +/+ mice survived at 30 dpi (($p \leq 0,01$). In accordance with this data, Tmem176b-deficient mice expressed higher levels of active IL1β ($p \leq 0,05$), and Caspase 1 ($p \leq 0,05$) in the liver. Also, increased levels MHV-RNA ($p \leq 0,05$), and elevated plasma GPT transaminase activity was detected ($p \leq 0,05$). Therefore, to verify the correlation between IL1β levels and survival, in vivo IL1β neutralization assays were performed. IL1β neutralization expands the survival of Tmem176b-deficient mice and liver viral load was significantly reduced. Although, this effect disappeared after simultaneous IL1β neutralization and CD8 T cell depletion. Thus, Tmem176b-deficient mice expand their survival after IL1β blockade in a CD8 T cells-dependent manner. In addition, flow cytometry analyses showed that Tmem176b-deficient mice had fewer MHV-specific CD8 T cells and showed lower MHV-specific CTL activity after in vivo CTL activity assay ($p \leq 0.01$).

Finally, to understand the mechanism behind this modulation, we analyzed the expression of the inhibitory receptor programmed death-1 (PD-1), on this model. Results showed that Tmem176b-deficient mice had an increased number of total CD8 T cells expressing PD-1 than wt mice. When PD-1 expression was analyzed in MHV-specific CD8 T cells, again Tmem176b-deficient mice showed augmented expression of this receptor.

To validate the relation between IL1β, PD-1 expression and survival, we performed anti-PD1 treatment on Tmem176b- deficient mice and observed that infected animals treated with anti-PD1 improved their survival rate to 57%. These results strongly suggest that IL1β is involved in survival to MHV infection and that inflammasome regulation is crucial to develop a proper antiviral immune response.

Conclusion: Collectively, we found that IL1β levels are crucial in the modulation of antiviral immune response through the modulation of PD-1 expression in virus-specific CD8 T cells in coronavirus-induced hepatitis.

Tmem176b is a checkpoint in IL-1β-dependent tumor immunity

Mercedes Segovia[1,2], Sofia Russo[1,2], Mathias Jeldres[1], Maria Cristina Cuturi[3], Marcelo Hill[1,2]

[1]Departamento de Inmunobiología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[2]Centre for Translational Immunology. FCE. Institut Pasteur de Montevideo, Montevideo, Uruguay
[3]INSERM UMR1064 Centre de Recherche en Transplantation et Immunologie, Nantes, France
msegovia@pasteur.edu.uy; russorossi.s@gmail.com; mathiasjeldres@pasteur.edu.uy; ccuturi@nantes.inserm.fr; mhill@pasteur.edu.uy

Keywords: Drug Discovery, Immunotherapy, Ion Channels, Inflammasome, tumor immunology

Tmem176b is an emerging immunoregulatory non-selective cation channel highly expressed in macrophages and DCs. We speculated that Tmem176b may promote tumor progression. In agreement with this hypothesis, we found that in a cohort of colon cancer patients (n=90), Tmem176b expression in the tumor infiltrate is significantly associated to diminished survival. Given this result, we were interested in determining the role of Tmem176b in cancer progression. We found that Tmem176b-/- mice had delayed tumor growth and better survival than WT mice when injected with two different transplantable cancer cell lines. Tumors from Tmem176b-/- were heavily infiltrated by tumor specific and total CD8+ T cells. CTLs depletion in Tmem176b-/- mice led to tumor development similar to the one observed in WT mice. In the tumor-draining lymph node (TDLN), CD4+Rorgt+ T cells were increased in Tmem176b-/- mice as compared to WT ones. IL-1β is known as critical cytokine to differentiate anti-tumoral Th17 cell. Active caspase-1 was increased in the TDLN from Tmem176b-/- mice as compared to WT. Moreover, Tmem176b-/- BMDCs showed significant higher IL-1β secretion upon different NLRP3 inflammasome activators than the WT ones. This was dependent on caspase-1 activation, K+efflux and cytosolic Ca2+release. Strikingly, in vivo IL-1β blockade in tumor-bearing Tmem176b-/- mice led to tumor development similar to the one observed in WT mice. In conclusion, Tmem176b impairs anti-tumoral immune responses by controlling IL-1β-dependent tumor immunity. Tmem176b may represent a new biomarker for cancer prognosis. We identified a pharmacologic Tmem176b inhibitor, which triggers tumor rejection depending on Tmem176b, caspase-1 and CD8+T cells.

Characterization of the Th2 environment and local IgE production in colorectal polyps of allergic patients

Karina Eva Canziani[1], Melisa Pucci Molineris[2], Norma Balcarce[3], David Díaz Jimenez[4], Luciana Guzman[3], Marcela García[3], Eugenia Margarita Altamirano[3], Dominik Meier[2], Marcela A. Hermoso[4], Cecilia Isabel Muglia[1], Guillermo Horacio Docena[1]

[1]Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina
[2]Favaloro University, Buenos Aires, Argentina
[3]Hospital de Niños Superiora Sor María Ludovica, La Plata, Argentina
[4]Universidad Autónoma de Chile, Santiago, Chile
karina.canziani@gmail.com; mpucci@ffavaloro.org; balcarcenorma@yahoo.com.ar; davordijim@gmail.com; guzman_155@hotmail.com; cmarcelagarcia@ciudad.com.ar; altamirano.eugenia@gmail.com; domeier@gmx.ch; marcehr@gmail.com; cmugliaenator@gmail.com; guillermo.docena@gmail.com

Keywords: IgE, Isotype switching, cow's milk allergy, Juvenile polyps, IL33/ST2

Juvenile polyps (JP) are common in children. They occur mainly single and typically at the sigmoid rectum portion. The most frequent presentation is painless rectal bleeding. JP contain proliferative lamina propria cells with abundant connective tissue, and a chronic cellular infiltration of eosinophils. However, the aetiology of JP still remains largely unknown and few reports have characterized the stroma of colorectal polyps in atopic patients. We previously showed that 70 % of patients older than 1 yo, with rectal bleeding and JP had high levels of serum IgE specific to cow milk proteins (CMP). This finding prompted us to study the relation between the aetiology of polyps and the atopic condition of patients.

Patients attending La Plata´s Children Hospital for rectal bleeding were subject to colonoscopy. Polyps (PT) and biopsies from sorrounding control tissue (SCT) were resected and studied. Blood IgE levels were simultaneously determined and children were routinely studied for allergy. Resected polyps from 18 patients were analyzed. Immunohistochemistry and confocal microscopy using anti-IgE, anti-CD138, anti-CD20, anti-ki67, anti-CD57, anti-AID, anti-IL33 and anti-ST2 conjugated antibodies were performed. Besides, cytokine mRNA expression of Ccl5, Ccl26, Il-17a, Ifn-γ, Il-13, Il-6, Il-4, Il-33, St-2s and St-2l were analyzed by RT-qPCR. Cytokine levels of different T helper profile were evaluated using CBA bead assay and ELISA. Germinal centers were isolated by dissection laser and analyzed by PCR to study the immunoglobulin class switching.

We found that 12 patients had serum CMP-specific IgE. The stroma of polyps showed a cellular infiltrate composed of mononuclear cells, mast cells, plasmatic cells and eosinophils with high levels of Ccl26 mRNA expression. IgE was found in plasma cells (30 ± 15 cells in 600 X fields) and eosinophils in P but SCT only showed a mononuclear cell infiltrate with IgA plasma cells but no eosinophils and a fewer number of IgE+ cells (2 ± 3 cells in 600 X fields). ST2+, IL-33+ and TSLP+ cells were also observed, confirming a Th2 infiltrate in polyps. Serum ST2 levels were higher in patients with polyps compared to control population (94 ± 15 vs 1233 ± 425 pg/ml, $p<0.05$). The analysis of mRNA expression showed a higher expression of il-4, il-33, ST2s ($p<0.05$) and a tendency to a higher expression of il-13 in PT than SCT, while ifn-γ and il-17 were diminished ($p<0.05$). At protein level, PT showed higher levels of IL-4, IL-5, IL-13 and lower levels of IL-17 and IFN-γ compared with SCT. The ratio IL-13/IFN-γ was increased in PT ($p<0.01$). PT also presented active germinal centers with CD20+, Ki67+, AID+, and CD57+ (TFH) cells and IgE+ plasma cells in the periphery. We demonstrated that IgE is produced through a sequential class switch (μ to γ and to ε).

In conclusion, we study for the first time the local immune signature of colorectal polyps in allergic patients. We found a Th2-associated inflammatory infiltrate in colorectal polyps with hyper eosinophilia and local production of IgE, which might be related to the atopic condition of patients.

In vitro alloantigen specific Tr1 differentiation and expansion for therapeutic use in kidney transplantion

Saúl Arteaga[1], Arimelek Cortés Hernández[1], Evelyn Katy Alvarez[1], Nadyeli Linares[1], Aurora Casillas[2], Josefina M Alberu[2], Eduardo Garcia[1], Soldevila Gloria[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
saul.arteaga.cruz@hotmail.com; arimelekch@hotmail.com; evelyn.alvarez.salazar@hotmail.com; nadyeli_linares@hotmail.com; auroracasillas.a@gmail.com; josefinaalberu@hotmail.com; garciaze@unam.mx; soldevi@unam.mx

Keywords: Kidney Transplantation, Transplantation Immunology, Tr1, differentiation and proliferation, Terapy

The main therapeutic alternative for patients that have lost the integrity and functionality in some of their organs is the organ transplantion. Currently, allograft rejection is controled by immunosuppressive drugs, however, these drugs increase the susceptibility to infections or the development of cancer. New therapeutic strategies involve the use of patients' immune cells with the capacity to suppress inflammatory responses. FoxP3+ regulatory T cells have been evaluated for this purpose, however the lack of specific surface markers has made difficult their identification and isolation. Recent reports have supported the use of Tr1 cells a subset of regulatory Tregs as one of the best candidates for use in new therapeutic protocols, based on their high production of IL-10 and because, unlike Foxp3+ Tregs, Tr1 cells are characterized by two specific surface molecules CD49b and LAG-3, which facilitates their identification and purification. Since the description of their specific phenotype, many researchers have focused their works on developing protocols to differentiate Tr1 cells in vitro, however, not all the protocols have been used in clinical trials. The "One Study" is an international organization responsible to evaluate the new improved protocols in clinical trials. The co-culture of allogenic DC10 and T naïve cells to differentiate in vitro allo-antigen specific Tr1 cells is a protocol approved in recent clinical assays for patients with the kidney transplant, showing good results to avoid rejection against the graft. DC10 are tolerogenic dendritic cells, which are characterized by their IL-10 production and the expression of co-inhibitory molecules ILT4 and HLA-G, which are good inducers for Tr1 differentiation.

Here, we performed allo-antigen specific Tr1 cells in vitro differentiation from naïve T cells by using allogenic DC10. PBMCs from healthy donors were purified by ficoll gradient and allogeneic monocytes were isolated by CD14 positive selection using magnetic beads. Then, they were stimulated for 8 days with IL-4 (50 ng/ml), GM-CSF (50 ng/ml), and IL-10 (50 U/ml) for DC10 in vitro differentiation, obtaining more than 80% of ILT4+/HLA-G+ cells. These cells were co-cultured with sorted CD4+ CD45RA+ CD25- allogeneic T naïve cells.

To assess the optimal Tr1 differentiation conditions, we evaluated the phenotype obtained by co-culture of DC10 with T naïve cells at different time points, in the presence of rhIL-10 and using different DC:T cell ratios. We observed a higher percentage of CD4+CD49b+LAG-3+ cells using 1:10 (DC10: T naïve) ratio in the presence of 50 U/ml of IL-10. We obtained a 30% of CD4+CD49b+LAG-3+ cells after 9 days culture with 95% of viability and this percentage increased at day 11 (37% with 80% of viability) reaching at day 14 the highest percentage of Tr1 cells (almost 80%) although the viability decreased to 40%. It is important to note that these double positive cells (CD49b+LAG-3+) among CD4+ T cells were not observed when T naïve cells are co-cultured with conventional dendritic cells.

Furthermore, we were able to expand the Tregs for at least 14 days after Tr1 differentiation, increasing 3 times total number. We also evaluated their suppressor function, by using autologous CD3+ cells, isolated by negative selection, as responder cells that were co-cultured with sorted Tr1 cells. Interestingly we observed that these cells were able to significantly suppress more than 40% of the proliferation of allogeneic CD4+T cells, but this effect was not observed on polyclonal T cells indicating that Tr1 cells are allo-specific. In summary, we have established an optimized protocol that allows efficient in vitro differentiation and expansion of Tr1 cells for their future therapeutic use in kidney transplanted patients. We are currently investigating the stability of these Tr1 under different inflammatory conditions.

Work was supported by CONACyT FOSSIS #272518 and Fundación Miguel Alemán. SA, AC, EA were recipients of fellowships from CONACyT.

Generation of large numbers of functional and stable human allo-specific induced-Tregs in the presence of pro-inflammatory cytokines

Saúl Arteaga[1], Evelyn Katy Alvarez[1], Arimelek Cortés Hernández[1], Nadyeli Linares[1], Aurora Casillas[2], Josefina M Alberu[2], Eduardo Garcia[1], Soldevila Gloria[1]

[1]Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
[2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico
saul.arteaga.cruz@hotmail.com; evelyn.alvarez.salazar@hotmail.com; arimelekch@hotmail.com; nadyeli_linares@hotmail.com; aurora.casillas@hotmail.com; josefinaalberu@hotmail.com; garciaze@unam.mx; soldevi@unam.mx

Keywords: Transplantation Immunology, Foxp3, Treg cells, expansion, Inmunotherapy

Regulatory T cells play an important role in the control of autoimmune diseases and maintenance of tolerance. Studies in animals and humans highlight their role in the induction of tolerance to allografts, which motivated the development of strategies to expand Tregs cells for therapeutic use. Although the clinical trials are focused at the use of Tregs cells of thymic origin as adoptive therapy, there are researches that support the use of in vitro induced-Tregs cells due to its superior functional stability and efficiency in inflammatory conditions, suggesting an advantage in a setting of allograft rejection. However, a problem of the in vitro induced-Tregs is the reported instability of their phenotype and methylation of the non-coding region of FOXP3 gene, although this may occur as a result of the methodological techniques used for their generation. For the above mentioned, we hypothesize that by improving the conditions of induction, it would be possible to achieve the desired phenotypic stability and in adequate numbers to be used in the clinical therapy.

The objective of this work is generate and expand allospecific Tregs, and assess their stability and suppressor function in presence of pro-inflammatory cytokines.

Dendritic cells were derived from monocytes (MoDCs) isolated from healthy voluntaries and were co-cultured with naive T cells from an unrelated individual, in presence of TGFβ1, IL-2 and Retinoic Acid. After 7 days of co-culture, proliferating CD25 HI cells (allo-specific induced-Tregs) were sorted and polyclonally expanded for 6 weeks in presence of TGFβ1, IL2 and Rapamycin. After 4 weeks of expansion, cytokines IL-6 and TNFα were added and the cultures were continued for two more weeks. The phenotype, suppressor function and production of cytokines were evaluated.

After 42 days of expansion, iTreg cells were expanded 2,300 times, giving rise to 4,600 millions of allo-specific induced Tregs from 20,000 initial naïve T cells. These iTregs may represent sufficient numbers to infuse several doses of up to 10x106 cells/kg per patient which is, on average, the reported in clinical trials. At this time, 88.5% of the expanded cells were CD4+CD25+FOXP3+, and their phenotype and expression of CD25/FOXP3 was preserved even in the presence of the pro-inflammatory cytokines TNFα and IL-6, suggesting that these cells are stable in an inflammatory environment and could be effective in a scenario of allograft rejection. Interestingly, allo-specific iTregs were able to specifically suppress the proliferation of autologous CD4 + and CD8 + T cells in response to the allo-MoDCs used for iTreg generation, but not to third party allo-MoDCs, and this suppression was not affected when the pro-inflammatory cytokines were added in the assay. In addition, iTregs were able to modulate cytokine production to exert suppression since it was observed a decrease of IL-2 and increase of IL-10 in the supernatants of the suppression assays, indicating that they may mediate suppression by IL-2 consumption and production of immunosuppressive cytokines. When TGFβ1 and/or Rapamycin were removed from the expansion cultures, iTreg cells showed a reduction in FOXP3 expression, as TGFβ1 alone was not sufficient to maintain the regulatory T cell phenotype, but with only rapamycin was sufficient to maintain the percentage of CD25+FOXP3+ and FOXP3 expression on the expanded iTregs. This result suggests that the use of in vitro generated allo-specific iTregs cells in adoptive therapy will require the use of Rapamycin to ensure their stability and efficacy. Although there are previous works on the generation of iTregs, this is the first report in which a large amount of iTregs are obtained and with specificity for the antigen of interest. To achieve this, the induction techniques were improved, including suboptimal activation of the TCR by allogeneic antigen recognition and the addition of of Retinoic acid and Rapamycin, with reported contribution to the stability of FOXP3 expression and suppressive function of Tregs. Currently, we are analyzing the epigenetic status of the TSDR region of FOXP3 gene from these cells, to give support to their stability and potential for use in adoptive therapy. In conclusion, a good number of allo-specific iTregs were induced with stable phenotype and antigen-specific suppressor function in presence of pro-inflammatory cytokines.

Work supported by CONACyT FOSSIS #272518, and Fundación Miguel Alemán, México. EA, AC and SA were recipients of fellowships from CONACyT.

CRTAM+ NK cells endowed with suppressor properties arise in leukemic bone marrow

Sandra Padilla-Castañeda[1,2], Dalia Ramirez-Ramirez[2,3,4], Carlos Samuel Galán-Enriquez[1], Jessica Lakshmi Prieto-Chavez[5], Elva Jiménez-Hernandéz[6], Armando Vílchis-Ordoñez[7], Juan Carlos Balandrán[1,2,3], Vianney Ortiz-Navarrete[1,3], Rosana Pelayo[2,3]

[1]*Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), México City, Mexico*
[2]*Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico*
[3]*FOCIS Centre of Excellence, Ciudad de México, Mexico*
[4]*Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico*
[5]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
[6]*Hospital Pediátrico Moctezuma, Moctezuma, Mexico*
[7]*Hospital Infantil de México Federico Gómez, Mexico City, Mexico*
sandy_pc@hotmail.com; dal2_r@hotmail.com; cgalane@cinvestav.mx; lakshmi.litmus@hotmail.com; elvajimenez@yahoo.com; vilchisarmando82@hotmail.com; jcbalandran@gmail.com; vortiz@cinvestav.mx; rosanapelayo@gmail.com

Keywords: NK cells, Acute Lymphoblastic Leukemia, tumor progression, CRTAM, suppressor-like NK cell

Background: Acute lymphoblastic leukemia (ALL), one of the major causes of pediatric mortality worldwide and the most frequent childhood malignancy, is characterized by uncontrolled oligoclonal proliferation of lymphoid precursors, mostly B lineage, within the bone marrow (BM), with relapses occurring in approximately 20% to 30% of cases due to minimal residual disease (MRD) and a failure in the mechanisms of cell-mediated antitumor immune surveillance. Natural killer cells (NK) are responsible for early responses mediated by the release of cytokines capable of lysing tumor cell. In humans, NK cells can be classified into two major sub-populations: the CD56dim CD16+ or cytotoxic NK cells (cNK) which predominates in peripheral blood (95% of NK) and sites of inflammation, and the CD56bright CD16- or regulatory (rNK) subset predominating in lymphoid nodes, which produces cytokines after activation and shows less cytotoxic capacity. Of note, a population of CD56+CD3- ILC with low cytotoxic capacity and production of IL-22, as well as a suppressor-like NK cell, characterized by producing IL-10 and TGFβ, have been recently found, although their precise identity stills to be determined. The abundance of a similar NK cell type, showing HLA-G+ IL-10+ TGFβ+ phenotype in peripheral blood of patients with breast cancer has been remarkable, suggesting a potential harmful role in antitumor immunity. NK receptors that regulate cell adhesion and activation through interaction with nectin or nectin-like ligands (Necl), including CRTAM originally described in activated NK,

NKT and CD8 cells. CRTAM expression is transient and peaks in membrane between 12 and 24h, followed by a decrease at 48h. Both, the receptor and its ligand Necl-2 are widely conserved between human and mouse and upon their interaction the regulation of cytokine production in CD4 T lymphocytes and the retention of CD8 T lymphocytes within lymph nodes are apparent. The interaction of CRTAM in NK, with its ligand Necl-2 expressed in tumor cells, promotes the cytotoxicity mediated by NK cells, whereas in CD8+ favors secretion of IFN-γ during a specific antigen stimulation of T cells. The existence of a subpopulation of NK cells whose expression of CRTAM suggests an anti-leukemic effector capacity is uncertain.

Objective: To determine the contribution of bone marrow CRTAM+ NK cells in early pro or antitumor responses in Acute Lymphoblastic Leukemia

Materials and Methods: Umbilical cord blood (UCB) samples were obtained from normal full-term neonates upon mothers' written informed consent, while normal bone marrow (nBM) aspirates were collected according to institutional guidelines from healthy pediatric donors who entered orthopedic surgery. Pathological BM aspirates were obtained from pediatric patients with newly diagnosed ALL. Peripheral blood (PB) samples were obtained from clinically healthy adult, while BM specimens were collected by aspiration before any treatment. Mononuclear cells (MNC) were obtained by Ficoll-Paque Plus. NK cells from PBMC and ALL were enriched using the Human NK Cell Isolation Kit or were purified by flow cytometry. CD34+ cells from MPB or UCB were enriched for most experiments using the Human CD34 Progenitor Cell Isolation Kit. Detection of membrane CRTAM in NK cells was achieved, upon activation, by flow cytometry using CD56, CD16 and CRTAM antibodies. The production capacity of IFN-γ, IL-10 and TGFb were analyzed. For the cytolytic activity of NK cells, the myeloid leukemia K562 cell line were stained with CTV and co-cultured with NK cells, according to an effector: target ratio of 10:1, respectively. Primary mesenchymal stromal cells (MSC) were obtained from normal or leukemic BM mononuclear cells. ALL or normal mononuclear cells were co-cultured with ALL or normal mesenchymal stromal. The analyzes were performed in NK or NKT for cell expression of CRTAM, IL-10, TGF-β and IFN-γ after the co-culture. Necl-2 gene was amplified in MSC, and the protein nectin-like-2 (Necl-2) investigated on the surface of BM stromal cells.

Results: The expression of CRTAM in cells of the immune system, including NK and CD8-T cells, has been related to its activation. Here, in vitro tests of NK CRTAM+ cells, mature and normal, revealed their cytotoxic and IFN-γ production capacity. The comparison of NK CRTAM+ and NK CRTAM- cells showed a similar cytotoxic

capacity, but higher production of IFNγ. In order to investigate CRTAM expression levels throughout hematopoietic development, we analyzed CD34+ cells enriched by columns. Expression of CRTAM was only apparent in the precursor stage and not in either, hematopoietic stem cells (HSC), multipotent progenitors (MPP) or early lymphoid progenitors (ELP), suggesting that the intrinsic differentiation program is accompanied by the display of activation molecules such as CRTAM, and discarding their substantial participation in the very primitive stages.

The CD56+CD3-CRTAM+ NK cell population was evaluated for its CRTAM expression levels, the highest expression was observed in NK CD56high cells, a presumably regulatory NK with poor cytotoxic capacity. When analyzing the expression of CRTAM in the group of patients with ALL, their NK cells express CRTAM with no ex vivo exposure to a stimulation factor. According to ours and others previous research, autologous or exogenous sources of pro-inflammatory factors may contribute the aberrant activation of committed bone marrow lymphoid or NK cell precursors during emergency hematopoietic conditions. Moreover, our results show a subpopulation of pre-activated NK CRTAM+ cells expressing IL-10 and TGFβ in the BM of patients with ALL.

Notably, leukemic stromal favors the expression of CRTAM in NK and NKT cells. Thus, gene transcription was investigated by RT-PCR in stromal cells of normal and leukemic BM, suggesting the possible CRTAM-Necl-2 interaction between NK or NKT cells and stromal cells BM niches. A substantial display of Necl-2 on stromal cell membranes, specially by ALL MSC, was found. Co-culture systems showed that leukemia stroma favors the expression of CRTAM, IL-10 and TGF-β, suggesting a contribution to the suppressor phenotype of NK cells in the leukemia setting.

Conclusion: Leukemic BM develops CD56high pre-activated CRTAM+ NK cells from B/NK lymphoid precursors residing in specialized niches expressing the natural ligand Necl-2. A novel CRTAM+ NK subset endowed with potential suppressive abilities is observed in leukemia setting that may favor tumor progression. Identify this unique NK population would be of special interest for the understanding of the pathobiology of ALL.

Immunoscoring in bone marrow aspirates as a novel tool for prognosis in acute leukemia patients

Gustavo Iñaki López Reyes[1,2], Blanca Félix Bañuelos Garcia[1], Lilia Adela García Stivalet[1], Jose Alejandro Limon[1], Juan Carlos Balandrán[1,3,4], Rosana Pelayo[1,4]

[1]*Instituto Mexicano del Seguro Social (IMSS) Delegación Puebla, Mexico, Puebla, Mexico*
[2]*Benemérita Universidad Autónoma de Puebla, Puebla, Mexico*
[3]*Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico*
[4]*Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico*
ponnylr@gmail.com; pipucho_sol@hotmail.com; lilia.garcia@imss.gob.mx; jose.limonf@imss.gob.mx; jcbalandran@gmail.com; rosanapelayo@gmail.com

Keywords: Acute leukemia, Bone marrow microenvironment, multiparametric flow cytometry, Cancer immune surveillance, Immunoscoring

Background and Aims: Acute leukemias are heterogeneous haematological disorders affecting lymphoid or myeloid precursors within the bone marrow. Despite the relative high remission rates, relapses often occur in patients, which has been attributed to the inability of current therapy of standard care to eliminate leukemia-initiating cells (LICs), capable of initiating and maintaining disease. Moreover, bone marrow (BM) microenvironment is believed to provide a protective niche for LICs, through regulatory signals essential for maintenance, proliferation and survival of malignant cells. We have previously documented the progressive competence for the normal niches leading to exhaustion of normal haematopoiesis. Low numbers of Hematopoietic Stem and Progenitor Cells (HSPC) is a hallmark in the various types of leukemia, with the worst prognosis and the lowest HSPC numbers exhibited by T-cell acute lymphoblastic leukemias. Moreover, very low contents of all innate and adaptive cell subsets, including natural killer (NK) cells, have been related to poor outcomes also. Routinely, immune cells are produced by early HSPC and some of them stay in BM to execute long term immune surveillance, which is necessary to detect and control malignant cells. In solid tumors, high numbers of immune suppressors cells and the lack of functional cytotoxic cells have been shown to associate to poor prognosis. Although it has been recognized that leukemic BM is a "bad neighborhood", little is known about the content, type and functional behaviour of the normal immunological residents. Treatment success must be promoted by contribution of immune cells capable of eliminating residual tumor cells during remission, but this has not been investigated in the leukemia setting yet. Because leukemias start and progress in BM where normal blood and immune cells are made, we propose an Immunoscore (IS) for leukemia patients, consisting of some

effector immune cell populations, including lymphocytes T CD4+ and T CD8+, Tregs, B cells, NK cells, NKT cells and myeloid cells in BM. A correlation analyses of such IS with the clinical data would reveal therapy effectiveness and/or immune reconstitution in oncological individuals.

Methods: We designed an antibody panel for multiparametric flow cytometry (11 parameters, 8 colors). Briefly, fluorochrome-labeled antibodies for CD45, CD19, CD3, CD4, CD8, CD56, CD14, CD11c, HLA-DR, Foxp3, and INF-gamma were used. 1x106 cells from BM aspirates from acute leukemia patients at clinical debut and follow-up of MRD were stained and flow cytometry was performed. Cytometry data analysis was conducted using FlowJo® and Infinicyt® softwares, while GraphPad Prism® software was used for statistics.

Results and Discussion: Nineteen leukemia patients were immunoscored, and normal mature B cells, T CD4+, T CD8+, Treg (Foxp3+), NK cells (CD56+CD3-), NKT cells (CD56+CD3+), Innate Lymphoid Cells (CD19-CD3-CD56+/-), Dendritic Cells (CD11c+), Granulocytes (SSC++CD45lowCD14+/-) and Monocytes (CD14++),as well as INF-gamma production were identified with our antibody panel in leukemia BM samples. As expected, essential immunological populations were decreased in all patients, particularly innate NK, ILC and granulocytes populations. Of note, very low numbers and frequencies of cytotoxic NK and T CD8+ cell subsets were recorded in MRD+ patients, while the Tregs pool tended to be higher when compared to those in clinical debut or in MRD negative leukemic conditions.

By inhibiting leukemic haematopoiesis, normal compartmental sizes of immune cells within leukemic BM should be able to restore the normal cell rate production and competent immunosurveillance. Our ongoing work suggests that the emergence of new malignant clones in the setting of minimal residual disease is related to poor functional immune surveillance mechanisms driven by regulatory or suppressor immune subsets. One mechanism involved in this phenomenon might include the unsuitable mesenchymal stromal cells microenvironment promoting an unbalance of effector and immunosuppressor populations.

Preliminar Conclusion

Immunoscoring of leukemia patients may help to reveal the real immunological status to control disease by immune effector cells. Investigating immunesurveillance cell contents at debut and upon minimal residual disease would be of substantial relevance

to control relapse through immunotherapy based on effector:suppression balance by strengthening the production of competent normal cells able to eliminate residual tumor, concomitant to inhibition of regulatory population. Thus, systematic bone marrow immunoscoring may provide a novel tool for Precision Oncology.

The Vm24 scorpion toxin blocks Kv1.3 potassium channels and attenuates the effector memory T cells response

José Ignacio Veytia-Bucheli[1,2,3], Juana María Jimenez-Vargas[2], Erika Melchy[2,3], Monserrat Alba Sandoval-Hernandez[1,2,3], Lourival Domingos Possani[2], Yvonne Rosenstein[1,2,3]

[1]Programa de Posgrado en Ciencias Bioquímicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
[2]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Ciudad de Mexico, Mexico
[3]Mexican Translational Immunology Research Group (MEXTIRG), Cuernavaca, Mexico
ignacioveytia@hotmail.com; jimenez@ibt.unam.mx; melchy@ibt.unam.mx; monseinm@ibt.unam.mx; possani@ibt.unam.mx; yvonne@ibt.unam.mx

Keywords: Autoimmune Diseases, Inflammation, Kv1.3 Potassium Channel, t effector memory cells, Vm24 toxin

In autoimmune diseases such as multiple sclerosis, type 1 diabetes mellitus, and rheumatoid arthritis, autoreactive T cells exhibit a TEM (effector memory) phenotype. In TEM cells, the ionic channel Kv1.3 regulates calcium signaling by potassium efflux, generating the electrochemical potential required for the entry of calcium through CRAC channels located in the plasma membrane. Calcium is essential for T cell activation, proliferation, migration and cytokine secretion and blockade of the Kv1.3 channels results in inhibition of the calcium influx, thus eliciting an immunomodulatory effect. Among the naturally occurring peptides, the 36 amino acid toxin Vm24 isolated from the Mexican scorpion Vaejovis mexicanus is the most potent (KD = 2.9 pM) and selective (>1500 times over other assayed potassium channels) Kv1.3 channel blocker known, which makes it a very promissory candidate for its use in the clinic for chronic inflammatory diseases treatment. To better characterize the molecular events that follow Kv1.3 blockade, we combined a proteomic analysis with a multiplex chemokine/cytokine assay on CD4+ TEM cells (CD4+, CD45RO+, CCR7-) isolated from healthy donors' peripheral blood, following TCR activation in the presence or absence of the Vm24 toxin. The peptide completely blocked Kv1.3 channels currents at a concentration of 1 nM without impairing TEM cell viability, and in response to TCR stimulation, it inhibited CD25 expression as well as the production of several pro- and anti-inflammatory cytokines. These results, in combination with data of the proteomic analysis, indicate that the biological processes most affected by the blockade of Kv1.3 channels in a T cell activation context were cytokine-cytokine receptor interaction, mRNA processing via spliceosome, response to unfolded proteins and intracellular

vesicle transport, suggesting an attenuation of the TEM cells protein synthesis machinery. As TEM cells are considered to be the main players in the pathology of autoimmune diseases, our results further support the search for specific Kv1.3 channel blockers such as the Vm24 peptide.

Wnt pathway regulators as biomarkers for diagnostic of rheumatoid arthritis

Mariela Gómez-Moreno[1,2], Christian Montoya-Vazquez[1,3], Julio Enrique Castañeda-Delgado[1,4], Martin Zapata-Zuñiga[1], Leonor Enciso-Moreno[1], Betzaida Cuevas-Córdoba[1], Noe Macias-Segura[1], Adriana Elizabeth Monsiváis-Urenda[2], Jose Alfredo Mendez[5], Jose Dionisio Castillo-Ortiz[6], Cesar Ramos-Remus[6], Jose Antonio Enciso-Moreno[1], Yadira Bastián[1,4]

[1]Unidad de Investigación Biomédica de Zacatecas (IMSS), Zacatecas, Mexico
[2]Facultad de Medicina, Universidad Autónoma de San Luis Potosi, San Luis Potosí, Mexico
[3]Universidad Autónoma de Zacatecas, Zacatecas, Mexico
[4]Cátedras CONACYT-IMSS, Zacatecas, Mexico
[5]Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico
[6]Unidad de Investigación en Enfermedades Crónico Degenerativas, Instituto Nacional de Enfermedades Respiratorias (INER), Guadalajara, Mexico
mgm_09_92@yahoo.es; chris_crazy@hotmail.com; julioenrique_castaeda@yahoo.com.mx; martinzz24@icloud.com; leonorenciso1@hotmail.com; betzaidacuevasc@gmail.com; roca148@hotmail.com; adriana.urenda@uaslp.mx; jamendez@mail.ifisica.uaslp.mx; drcastilloortiz@gmail.com; r_ramos@prodigy.net.mx; enciso_2000@yahoo.com; ybastianhe@conacyt.mx

Keywords: Wnt Proteins, biomarkers, DKK1, SOSTDC1, WIF1

Introduction: Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology. This disease is associated with a chronic inflammation of the joints, pannus formation, loss of cartilage and bone reabsorption . One of the characteristics of RA is the proliferation of Fibroblasts-like synoviocytes (FLS) within the synovial membrane, which is induced by the activation of the Wnt signal pathway. Furthermore, activation of this pathway in FLS is involved in the production of several cytokines as well as of metalloproteinase 3, promoting cartilage degradation[1],[2].

Wnt signalling is regulated through multiple mechanisms, including extracellular regulators that can decrease the activity of the pathway. Among them, Dickkopf 1 (DKK1) is one of this regulators that has been related to several musculoskeletal diseases such as osteoarthritis, ankylosing spondylitis[3] and RA[4]. Other regulators are Sclerostin and Wise, which as DKK1, have the ability to bind to Wnt receptors, specifically to LRP6, thus preventing the binding of Wnt proteins.

In order to establish future biomarkers for the early diagnosis of RA, the goal of this work was to analyze the gene expression of several regulators of the Wnt signal pathway during the establishment of RA.

Materials and Methods: Patients and sample collection. Whole blood were collected and 1ml of RNAlater (Invitrogen) was added. Samples were immediately stored at -70°C until processed for RNA extraction. All RA patients fulfilled the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR). Patients were classified in two groups: early RA (less than a year with symptoms) and chronic or established RA (more than one year with symptoms). Also, First-degree relatives of RA patients were categorized into two groups: those negative for ACCP (ACPA-) and positive for ACCP (ACPA+).

Real Time PCR. Using Sso Fast-EvaGreen® (Bio Rad). Real Time-qPCR was then performed for the determination of the gene expression values. HPRT was used as reference gene, (Supp 1) Statistics. RT-qPCR data was analyzed with non-parametric test were carried out in GraphPad Prism 5.0 (GraphPad Software, Inc, USA) and SPSS version 18 (Microsoft, USA).

Results: Table 1 shows both demographic and serological parameters of all subjects in this study. Briefly, the values of ACPA and RF antibodies were higher in both RA groups, when compared to the group of healthy relatives (both ACPA+ and ACPA-). However, we did not found differences in anti-CarP antibodies between all groups.

Table 1: Demographics and serological parameters of the studied population.

	Relatives of patients with ACCP negative	**Relatives of Patients with ACCP positive**	**Early RA**	**Chronic RA**
Age Mean (±SD)	39.9 (15.48)*	37.7 (9.8)*	41 (12)*	55.7 (9.9)*
Gender (F/M)	17/3	17/3	18/2	19/1
Values of ACCP Median (IQR).	13.5 (12.7-15.6)	26.9 (27.2-33.2)	180.5 (23.7-683.3)*	391.5 (158.4-978.7)*
Values of Anti Carb-P Median (lOR).	162.1 (147.1-186.8)	155.6 (122.8-184.3)	179.0 (90.5-314.4)	244.6 (147.4-498.8)
Values of FR Median (IQR).	4.8 (2.9-6.2)	6.3 (2.2-7.7)	171.3 (82.95-367)*	160 (78.4-178.4)*

SD=Standard Deviation; F/M=female/male; IQR=interquartile range. All groups have differences in the age range. The median values of the ACCP levels in Early RA and Chronic RA from the healthy donors. The values of the Anti Carb-P antibodies do not have differences there between.

The Wnt signal pathway has been involved in RA and the serum levels of DKK1 and Sclerostin have been evaluated indeed. Of these two regulators, only DKK1 is increased in this pathology. In spite of this, the gene expression levels of DKK1 and other regulators of the Wnt signal pathway had not been assessed in eRA or in first-degree relatives at low- (ACPA-) or high risk (ACPA+).Therefore, in order to get information about the regulators of Wnt, the expression of DKK1, SOSTDC1 and WIF1 genes was evaluated. No significant differences were found for DKK1 among all groups (FIGURE 1A). However, significant differences were found for SOSTDC1 and WIF1 when their expression was compared between ACPA- and eRA or cRA as well as between ACCP+ and eRA (FIGURE 1B and 1C respectively).

We next evaluated whether SOSTDC1 or WIF1 have any power to discriminate RA patients from healthy subjects (first-degree relatives, either ACPA+ or ACPA-).ROC curves were performed and the AUC for SOSTDC1 was of 0.881 whereas for WIF1 was of 0.798. Sensitivity and specificity for these genes were evaluated and cut-off points allowing a balance between sensitivity and specificity were taken(FIGURE 2 and dashed line in FIGURE 1B and 1C).

In order to determine any correlation between the expression of the regulators and the presence of ACPA or anti-CarbP antibodies, we performed a non-parametric Spearman correlation analysis. We identified a strong positive correlation between SOSTDC1 and WIF1, and another of both genes with DKK1. In addition, we also found negative correlations between SOSTDC1 and WIF1 with the anti ACPA antibodies.

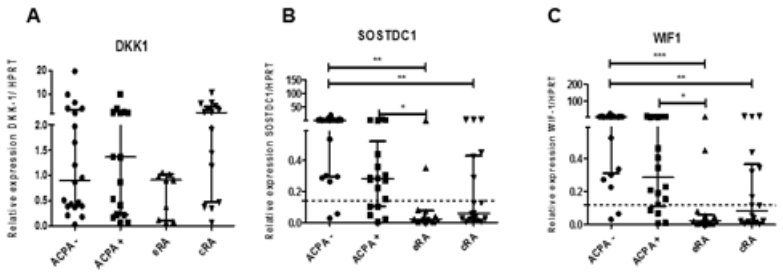
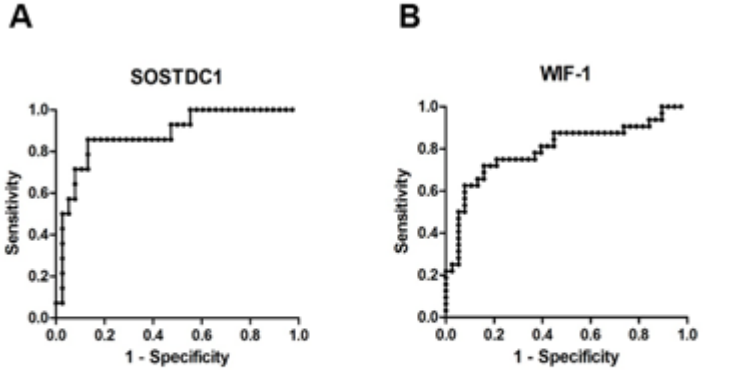


FIGURE 1: PCR results of gene expression of DKK1, SOSTDC1 and WIF1.
ACPA-: Direct relatives of the RA patients with levels minor to 25 AU of ACPA, ACPA+: Direct relatives of the RA patients with levels higher to 25AU of ACPA, eRA: early RA, cRA: chronic RA, *= p<0.05, **= p<0.01, ***=p<0.001.



C

Gene Name	AUC	Asymptotic significance	Point off cut	Sensitivity	Specificity
SOSTDC 1	.881	<.0001	<0.1278	.8571	.8158
WIF 1	.798	<.0001	<.1182	.718	0.842

Figure 2: ROC curve of SOSTDC1 and WIF1.

Discussion: As already mentioned above, one of the regulators analyzed in the present work, DKK1, has been previously measured in serum of RA patients. However, there is still some controversy. Some studies have indicated that an inflammatory microenvironment can enhance its production, on the opposite, some authors have shown a decrease[4],[6]. Contrary to those reports, we did not find any significant difference between groups. Yet, in this study we provide evidence for the association of the expression of Wnt regulators with RA. The expression of both regulators, SOSTDC1 and WIF1, is decreased in RA, as result of this, our data suggested that in RA the Wnt signal pathway is up regulated, and thus likely promoting inflammation and hyperplasia [7, 8] .Moreover, our results show, for first time, that SOSTDC1 and WIF1 could be used as biomarkers of RA. However, opposite to what has been described for DKK1, these regulators are down regulated in the disease.

ACPA autoantibodies have a strong association with the development of RA as a significant correlation has been found between their production and the presence of the HLA-DRB1 risk alleles[9],[10].Moreover, for diagnosis, ACPA autoantibodies have a sensitivity of 72% and a specificity of 92%[11],[12]. Here, we show a correlation between ACPA autoantibodies and SOSTDC1 and WIF1.We thus propose that evaluation of the expression levels of these genes could add to the diagnosis of RA at early stages. In addition, the correlations we found between the expression of these regulators

Table 2: Correlations between gene expression and autoantibodies levels.

Gene		DKK1	SOSTDC1	WIF1	ACPA	Anti Carb-P
DKK1	Correlation	1.000	**.402****	**.484****	.035	.016
	Coefficient					
	Significance		.001	.000	.787	.901
SOSTDC1	Correlation		1.000	**.930****	**-.417****	.012
	Coefficient					
	Significance			.000	>0.001	.922
WIF1	Correlation					
	Coefficient			1.000	**-.358****	-.003
	Significance				.002	.982

*=p<0.05, **=p<0.01, the correlations in **bold** are strong and significant.

suggest that they could be used for a better discrimination of RA patients from healthy subjects. Moreover, these correlations imply a relationship de co-expression between these genes. Recently, Holdsworth and cols. described that administration in mice of antibodies against Sclerostin increases the expression of WIF1 and DKK1, thus showing that Sclerostin down regulates these genes[13].Finally, we found a negative correlation between the expression of either SOSTDC1 or WIF1 and the levels of ACPA autoantibodies, which may indicate a down regulation of these regulators due to the presence of autoantibodies. Although this effect may not be so direct as antibodies are unlikely to directly interact with Wnt receptors, this may involve indirect pathways of regulation.

REFERENCES

1. PH, R.-d.-C., et al., Rheumatoid arthritis: Nuclear Medicine state-of-the-art imaging. World J Orthop, 2014. 5: p. 312-318.
2. Sen, M., Wnt signalling in rheumatoid arthritis. Rheumatology, 2005. 44(6): p. 708-713.
3. Daoussis, D., et al., Evidence that Dkk-1 is dysfunctional in ankylosing spondylitis. Arthritis & Rheumatism, 2010. 62(1): p. 150-158.
4. Seror, R., et al., Increased Dickkopf-1 in Recent-onset Rheumatoid Arthritis is a New Biomarker of Structural Severity. Data from the ESPOIR Cohort. Scientific Reports, 2016. 6: p. 18421.
5. Zhang, L., et al., Serum DKK-1 level in the development of ankylosing spondylitis and rheumatic arthritis: a meta-analysis. Experimental & Molecular Medicine, 2016. 48(4): p. e228.
6. Stock, M., et al., Wnt Inhibitory Factor 1 Deficiency Uncouples Cartilage and Bone Destruction in Tumor Necrosis Factor α–Mediated Experimental Arthritis. Arthritis & Rheumatism, 2013. 65(9): p. 2310-2322.
7. Sen, M., et al., Expression and function of wingless and frizzled homologs in rheumatoid arthritis. Proceedings of the National Academy of Sciences of the United States of America, 2000. 97(6): p. 2791-2796.
8. Sen, M., et al., Blockade of Wnt-5A/Frizzled 5 signaling inhibits rheumatoid synoviocyte activation. Arthritis & Rheumatism, 2001. 44(4): p. 772-781.

9. Laki, J., et al., Very high levels of anti–citrullinated protein antibodies are associated with HLA–DRB1*15 non–shared epitope allele in patients with rheumatoid arthritis. Arthritis & Rheumatism, 2012. 64(7): p. 2078-2084.
10. Okada, Y., et al., Risk for ACPA-positive rheumatoid arthritis is driven by shared HLA amino acid polymorphisms in Asian and European populations. Human Molecular Genetics, 2014. 23(25): p. 6916-6926.
11. Padyukov, L., et al., A gene–environment interaction between smoking and shared epitope genes in HLA–DR provides a high risk of seropositive rheumatoid arthritis. Arthritis & Rheumatism, 2004. 50(10): p. 3085-3092.
12. Aggarwal, R., et al., Anti-Citrullinated Peptide Antibody (ACPA) Assays and their Role in the Diagnosis of Rheumatoid Arthritis. Arthritis and rheumatism, 2009. 61(11): p. 1472-1483.
13. Holdsworth, G., et al., Dampening of the bone formation response following repeat dosing with sclerostin antibody in mice is associated with up-regulation of Wnt antagonists. Bone. 107: p. 93-103.

Targeting EGFR in cancer patients: passive and active therapy

Zaima Mazorra[1], Anabel Lavastida[1], Lazaro Aira[1], Iraida Caballero[2], Fernando Concha-Benavente[3], Ivan Cuevas[4], Carlos Frometa[4], Patricia Lorenzo-Luaces[1], Belinda Sánchez-Ramírez[1], Angel Casaco Parada[1], Robert Luois Ferris[3], Tania Crombet[1]

[1]Center of Molecular Immunology (Cuba), Havana, Cuba
[2]Hermanos Ameijeiras Hospital, Havana, Cuba
[3]Hillman Cancer Center, Pittsburgh, United States
[4]Instituto Nacional De Oncología Y Radiobiología (INOR), La Habana, Cuba
zaima@cim.sld.cu; alavastida@cim.sld.cu; lazaroaira210786@gmail.com; iraida.caballero@infomed.sld.cu; conchabenaventef@upmc.edu; icuevasv@infomed.sld.cu; cfrometan@infomed.sld.cu; patricial@cim.sld.cu; belinda@cim.sld.cu; casaco@cim.sld.cu; ferrrl@UPMC.EDU; taniac@cim.sld.cu

Keywords: Safety, T cells, prostate cancer, monoclonal antibodies, head and neck cancer, Natural Killer cells, Therapeutic vaccine, Immunogenicity, Human epidermal growth factor receptor

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of four structurally related receptor tyrosine kinases. Ligand binding causes dimerization of the receptor which triggers a signaling cascade that promotes cell survival and tissue invasion, while inhibiting apoptosis. EGFR is frequently overexpressed in epithelial malignancies, which corresponds to a decrease in patient survival, providing a target for tumor antigen (TA)-targeted monoclonal antibody (MAb) and cancer vaccines-based immunotherapy. An anti-EGFR MAb named nimotuzumab and a EGFR-based vaccine have been developed at the Center of Molecular Immunology in Cuba.

On one hand, the EGFR vaccine consisted of the extracellular domain of the human EGFR molecule and very small size proteoliposome (VSSP) from Neisseria meningitis and Montanide ISA-51 as adjuvants. Castration- resistant prostate cancer patients were included in five groups according to the vaccine dose (100 μg, 200 μg, 400 μg, 600 μg and 800 μg) and vaccinated intramuscularly during 6 months. The primary endpoints were safety and immunogenicity. No grade III or IV adverse events were reported. High titers of anti-EGFR antibodies were observed in most of the evaluated patients. Only patients receiving the higher doses of vaccine showed significant tumor cell recognition and EGFR phosphorylation inhibition by hyperimmune sera. Forty percent of the patients showed a specific T cell response against EGFR peptides pool in post-treatment samples. Despite the small amount of patients, encouraging clinical results have been obtained. Interesting, there was an association between the immune response and the survival of the patients (1).

On the other hand, nimotuzumab is a humanized monoclonal antibody (MAb) for the treatment of EGFR over-expressing tumors and has been used with success in advanced unresectable locoregional SCCHN patients. The survival advantage and long-term duration of response seen after six weeks of therapy with nimotuzumab suggested that inhibition of EGFR signal transduction and tumor proliferation are not the only effector mechanism involved. Recently, we published for the first time, that nimotuzumab can induce NK cell–mediated ADCC at similar levels than cetuximab, despite its lower affinity for the EGFR. Nimotuzumab-activated NK cells promoted DC maturation and EGFR specific CD8+ T cell priming. Interestingly, this MAb led to upregulation of some immune checkpoint molecules on NK cells (TIM-3) and DC (PD-L1), to a lower extent than cetuximab. Notably, circulating EGFR-specific T cells were identified in long-term nimotuzumab-treated SCCHN patients. Besides, nimotuzumab combined with cisplatin-based chemotherapy and radiation increased the frequency of peripheral CD4+CD39+FOXP3+ Tregs which otherwise were decreased to baseline values when nimotuzumab was used as monotherapy (2).

The detection of specific T cells in both types of anti-EGFR treatments paves the way to design clinical trials combining passive and active immunotherapies. In addition, the combination with anti-checkpoints inhibitors could be an alternative for increasing the frequency of effective T cells against the tumor and consequently prolonging the survival of the patients.

REFERENCES

(1) Front Pharmacol. 2017 May 10;8:263. doi: 10.3389/fphar.2017.00263. eCollection 2017.

(2) Front Pharmacol. 2017 Jun 19;8:382. doi: 10.3389/fphar.2017.00382. eCollection 2017.

Immunopaedia: The worldwide immunology learning website

Cristina Artaza-Irigaray[1], Jennifer Enciso[2,3,4], Juan Carlos Balandrán[3,5], José Luis Maravillas-Montero[6], Cheleka Anne-Marie Mpande[7], Thandeka Moyo[7], Bonamy Karushka Holtak[7], Michelle Letarte[8,9], Clive Maurice Gray[7]

[1]División de Inmunología, Centro de Investigación Biomédica de Occidente (CIBO), Guadalajara, Mexico
[2]Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México, México City, Mexico
[3]Centro de Investigación Biomédica de Oriente (CIBIOR), Puebla, Mexico
[4]Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Mexico City, Mexico
[5]Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico
[6]Red de Apoyo a la Investigación (RAI), Ciudad de México, Mexico
[7]Division of Immunology, University of Cape Town, Cape Town, South Africa
[8]Department of Immunology, University of Toronto, Toronto, Canada
[9]Molecular Medicine Program, Hospital for Sick Children, Toronto, Canada
chispuncris@hotmail.com; jenny_gr8@msn.com; jcbalandran@gmail.com; maravillas@cic.unam.mx; mpnche001@myuct.ac.za; thandeka.moyo@uct.ac.za; bon.holtak@uct.ac.za; michelle.letarte@sickkids.ca; clive.gray@uct.ac.za

Keywords: open-access, Clinical Immunology, educational resources, basic immunology, Immunopaedia

The improvement in research tools and technologies has led to a growing scientific knowledge and there is a need to gather all the information in trustful open-access websites. Regarding the immunology field, a non-profit educational website was launched in 2005 in South Africa and has become a broad resource of immunological knowledge and clinical studies targeted at researchers, healthcare professionals and students.

Immunopaedia works in partnership with the International Union of Immunological Societies (IUIS) and offers high quality online courses for participants to immunology courses and conferences all over the world. Currently, the development of Immunopaedia is overseen by teams based at the University of Cape Town (South Africa) and University of Toronto (Canada) in conjunction with a Steering Committee composed of 12 immunology researchers and lecturers from different universities around the world.

The webpage provides educational materials divided in sections on the basics of the immune system as well as advanced immunology. Moreover, the website contains

clinical case studies to complement the theoretical knowledge. Finally, Immunopaedia supplies information on treatment and diagnostics with a focus on infectious diseases. Apart from the educational resources, every month, Immunopaedia features an interview with an immunologist that is at the cutting-edge of research and education. The latest news on immunology research are also published.

Every scientist, student and teacher on the immunology field is strongly encouraged to use the materials and resources supplied by the Immunopaedia webpage both for learning and teaching, as well as for future working collaborations.

Links to access Immunopaedia:
www.immunopaedia.org.za
www.facebook.com/immunopaedia
Twitter: @Immunopaedia

Personalized bone marrow organoid-like system to predict chemoresistance to antineoplastic drugs in acute leukemias

Jiovanni Amador Cardoso[1,2], Lilia Adela García Stivalet[1], Jose Alejandro Limon[1], Rosana Pelayo[1], Juan Carlos Balandrán[1,3]

[1]*Instituto Mexicano del Seguro Social (IMSS), Mexico City, Mexico*
[2]*Benemérita Universidad Autónoma de Puebla, Puebla, Mexico*
[3]*Departamento de Biomedicina Molecular, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico, Mexico*
jiovanni-amador@hotmail.com; lilia.garcia@imss.gob.mx; jose.limonf@imss.gob.mx; rosanapelayo@gmail.com; jcbalandran@gmail.com

Keywords: Acute leukemia, chemoresistance, personalized oncology, tumor microenvironment (TMA), bone marrow organoids

Introduction: Acute leukemias (AL) is the major cause of death in pediatrics in Latin America as well as worldwide. These malignant neoplasms are characterized by the uncontrolled production of dysfunctional lymphoid or myeloid precursors in the bone marrow (BM). Its etiology is subject of intense research where leukemia-initiating cells (LIC) have been shown to be responsible for chemoresistance and further relapse. The progress in the integral management of the disease has been remarkable in the last decades, contributed by international cooperation efforts and the partial understanding of their complex pathobiology, including genetic and epigenetic lesions associated with cellular transformation. Despite the high remission rates (~ 80%), chemoresistance jeopardizes the success of treatment with subsequent relapse in more than 20% of patients, which is attributed to the inability of chemotherapeutic regimens to eliminate cells LIC. The design of new treatments has played a crucial role in this evolution. There are four treatment phases / protocols based on the guidelines national and international: the induction to remission, which aims to eradicate the signs and symptoms of the disease and restore normal hematopoiesis, blasts reduction in BM to less than 5%, absence of blasts in cerebrospinal fluid and minimal residual disease less than. Ideally, this phase lasts six weeks and may include the following medications: dexamethasone, prednisone, vincristine, daunorubicin, L-asparaginase, etoposide and arabinoside cytosine. Preventive therapy of CNS leukemia, which uses intrathecal metrotexate. Therapy of consolidation, this phase is continuous to that of induction, has the objective of intensifying the treatment using high doses of antimetabolites such as AraC, mercaptopurine, cyclophosphamide and methotrexate. Continuation or maintenance therapy, with 6 mercaptopurine and metrotexate, lasts two or three years and its objective is to eliminate any remnant (residual) leukemia cells. Resistance to chemotherapy can be

innate or acquired, and multiple biological mechanisms, including reduction in drug uptake or expulsion, intrinsic factors and metabolic state of the tumor cells, increase of the active flow of hydrophobic drugs that enter the cell, etc. The final result in any case is the permanence of the clones aberrant and sustained leukemic hematopoiesis with poor remission capacity. In normality, the production of cells of blood lineages is a highly regulated process starting in stem cells and progenitors cells (HSPC). Neither leukemic blasts or HSPC can live independently of their niches, which offers them dynamic interactions and regulatory signals essential for its maintenance, proliferation and survival. As AL are a group of diseases that begin and progress in the BM and considering that the complex three-dimensional structure of this organ is decisive for the organization of specialized niches, the integral study of this pathology requires the understanding of the repercussions of this communication on malignancy. During disease, leukemic cells hijack normal niches to create optimal sanctuaries to hide away from of therapy. Conventional co-cultures with primary BM stromal cells cannot be totally supportive for in vitro leukemogenesis, suggesting the high dependence with the natural BM microenvironment. Experimental evidence in our lab have shown the benefits to implement a BM organoid-like model that mimics better the tumor microenvironment in a three-dimensional (3D) architecture simulating ex vivo the architecture of the BM and the interaction microenvironment-leukemia-initiating cells (LIC)-blast, favoring the differentiation and maintenance of leukemic precursor. When compared to bidimensional cultures using stromal monolayers (2D), the capacity of LIC in xenotransplantation in vivo model is substantially increased. In addition, the system distinguishes blasts leukemic by its tropism by external areas, more primitive cells CD34+ migrates preferentially to internal areas. Here, we hypotheses that a high-throughput scanning (HTS) will provide personalized information about drug sensitivity and/or resistance in leukemia patients contributing to the optimization of their treatment to prevent early relapse.

Materials and Methods: OP9 stromal cells and primary human BM stromal cells were induced to form 3D multicellular spheroids (organoids). Mononuclear cells were obtained from BM aspirates samples from leukemia patients at their clinical debut and were co-cultured with the stromal organoids, stromal monolayers and stromal-free conditions with drugs from the standard care at different doses and combination. Viability assays were performed with flow cytometry and fluorescent microscopy by using fluorescent viability dyes (DAPI, 7-AAD) after 24 and 48 hours of treatments. Implementation of customized platforms will allow multiple evaluation of doses and combinations of drugs used for the induction of remission. Changes in cell frequencies, phenotype, proliferation and apoptosis of leukemic and stromal cells will be monitored. The follow-up of the patients will determine the correlation of the personalized test with clinical evolution.

Preliminary Results and Discussion: We have standardized co-cultures with BM organoids using leukemic cell lines Nalm-6, Reh and RS4;11. To demonstrate drug diffusion into the system, organoids were recovered after leukemic colonization and incubated with fluorescent dyes and drugs prior to being enzymatically digested. Reh and Nalm-6 (4,5% and 3%, respectively) were better organoid-colonizer than RS4;11 (1%). Data shown that stromal cells as well as inner leukemic population can incorporate dyes/drugs by analyzing CD45+ cells by flow cytometry. Using a HTS platform, cell viability after vincristine, dexamethasone and prednisolone exposition was recorded on several culture conditions: stromal-free (SF), 2D and 3D. As previously reported, combination of drugs was more efficiently in killing ALL cells when compared to individual effects. Alone, Vincristine resulted more cytotoxic than prednisolone or dexamethasone. Interestingly, Reh cell line were naturally more resistant (~50% higher) in SF culture conditions. 2D co-cultures with OP9 stromal cells increases the viability (~45-60%) of all leukemic cells tested compared to SF conditions. Analyses of 3D co-cultures suggested that viability of leukemic cells living outside the organoids is slightly higher than in 2D scenarios. However, 85-96% of B-ALL viable cells are recovered from inner structures even upon treatment with the highest concentrations of drug combinations. As known, chemoresistance is highly promoted by stromal cells but it is enhanced in our organoid-like model. Interestingly, Nalm-6 resistant cells can survive to high doxorubicin doses when the inner cells in the organoids were analyzed. A secondary drug exposition without stromal cells affected only 50% of leukemic population, suggesting that chemorresistant phenotype may be induced

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership