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REVISTA CUBANA DE

FARMACIA

Volumen 42 (Suplemento Especial No. 1), Año 2008

PROGRAM AND ABTRACTS

IMMUNOPHARMACOLOGY 2008

1st International Workshop of ImmunoPharmacology
5th International Workshop of Inflammation and Pain,
1st International Workshop of NeuroImmunology,
1er International Symposium about Pharmacology of Cytochrome P450.

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Dr. Silvio Perea Rodríguez.

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Dra. Idania Rodeiro Guerra.

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Volumen 42 (Suplemento Especial No. 1), 2008

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El Presente Número constituye un Suplemento Especial que recoge los trabajos presentados en el IV Congreso Nacional de Farmacología y Terapéutica.
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Admitimos contribuciones de profesionales cubanos y extranjeros. Los originales deben ser remitidos según las "Instrucciones al Autor" Los trabajos serán inéditos. Solicitamos y agradecemos el canje con publicaciones similares.

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Cuban Society of Pharmacology

1st Internacional Workshop of ImmunoPharmacology

5th Internacional Workshop of Inflammation and Pain

1st International Workshop of NeuroImmunology

1st International Symposium about Pharmacology of Cytochrome P450

IMMUNOPHARMACOLOGY 2008

ABSTRACTS

Plaza América Convention Center & Club Amigo Hotel
Varadero, Cuba

April 19-22, 2006

http://www.scf.sld.cu/impharmacology08/impharmacology08.htm

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PREFACE

The Cuban Society of Pharmacology calls its associates and professionals related with the pharmacological sciences' work at international level to the celebration of the 1st International Workshop of ImmunoPharmacology, the 5th International Workshop of Inflammation and Pain, the 1st International Workshop of NeuroImmunology and the 1st International Symposium about Pharmacology of Cytochrome P450 (ImmunoPharmacology 2008).

This congress constitutes an exceptional opportunity to deepen in the knowledge, to strengthen the scientific exchange and the collaboration among professionals that develop their daily activity in the field of the pharmacological sciences and other related disciplines, in function of immunology, inflammation, pian, neurology and cytochrome P450. Around 100 Cuban researchers, coming from universities, faculty of medical sciences, investigation institutes, assistance centers, hospitals and community pharmacies participate in this event. This represents a qualitative jump for the Cuban Society of Pharmacology, when being a forum framed in these specific topics. All of this shows the importance to the realization of these scientific activities as part of the strategies that are developed in the country to carry out the integration, collaboration and the professionals' scientific update those that work on this important field of the pharmacological investigations.

It is necessary to highlight the participation of more than 100 scientific personalities from Argentina, Australia, Belgium, Brazil, Cameroon, Canada, China, Colombia, Czech Republic, Gambia, Germany, Iran, Macedonia, Mexico, Netherland, Nigeria, Norways, Peru, Spain, Sudan, Taiwan, Turkey, United States, United Kingdom and Venezuela, who with their presence in our country, the quality of the sent works and their continuous support messages and solidarity toward our scientists, our people and, in particular, to the organizational work of this Forum, already leave an indelible print for the history of these scientific events.

About 200 works among plenary lectures, lectures and presentations in posters; as well as the debate, the analysis and reciprocal exchange will give relief to this activity. They also will allow transferring to the participant's institutions in more than 20 countries the acquired knowledge, the need to continue in the search of new solutions and the necessary increment of the specialist's paper in pharmacology and other sciences in the development of new medicines and knowledge.

Welcome to everybody to the wonderful scenario of these Congress, the most beautiful beach in Cuba, Varadero. We are convinced that this meeting will allow creating new perspectives for the future work and it will consolidate even more the scientific activity of all the participants here congregated.

Thank you to all for your participation.

Organizing Committee

ImmunoPharmacology 2008

IMMUNOPHARMACOLOGY 2008
1st International Workshop of ImmunoPharmacology
5th International Workshop of Inflammation & Pain
1st International Workshop of NeuroImmunology
1st International Symposium about Pharmacology of Cytochrome P450
Varadero, Cuba. April 19-22, 2008
http://www.scf.sld.cu/impharmacology08/impharmacology08.htm

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1st International Workshop of NeuroImmunology

1st International Symposium about Pharmacology of Cytochrome P450

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Posters of Inflammation & Pain (PIF)

MONDAY, APRIL 21

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PROGRAM

Saturday,

April 19

09:00-14:00 **Registration.**

Club Amigo Varadero Hotel.

17:00-17:30 **Opening Ceremony**

At Plaza America Convention Center, Varadero beach, Cuba.

17:00-17:15 Introductory lecture. **René Delgado Hernández, President of Cuban Society of Pharmacology.**

17:15-17:30 Presentation of the Congress Program. **Gabino Garrido Garrido, President Scientific Committee.**

17:30-20:00 **Opening Lectures**

17:30-18:15 **PL-01:** The autoimmune pathogenesis of multiple sclerosis. **Hartmut Wekerle, President of International Society of Neuroimmunology, ISNI (Germany)**

18:20-19:05 **PL-02:** Drug-induced idiosynchratic hepatic reaction. **Jose Vicente Castell Ripoll (Spain)**

19:10-19:55 **PL-03:** Immunotherapy of cancer: More than just antibodies and vaccines. The importance of synergy and sequential management in cancer. **Angus Dagleish (UK)**

20:30-21:30 Return to Club Amigo Hotel

Dinner (Only for delegates logged at Club Amigo Varadero Hotel)

21:30-23:50 **Welcome Cocktail in Club Amigo Varadero Hotel**

Cultural activities, Music & dancing.

Revista Cubana de Farmacia vol. 42 (Suplemento 1):1, 2008

Sunday, April 20 **ImmunoPharmacology 2008**

At Plaza America Convention Center, Varadero beach, Cuba.

9:00-11:00 **Lectures**

Chairs: Sylvie Marleau (Canada) and Circe Mesa (Cuba)

9:00-9:35 **PL-04:** Combining suppressive and immuno-stimulatory signals on proteoliposomes: a new approach for immune compromised scenarios. **Circe Mesa (Cuba)**

9:35-10:10 **PL-05:** CD36: a target for the anti-inflammatory and anti-atherosclerotic properties of growth hormone-releasing peptides (GHRPs). **Sylvie Marleau (Canada)**

10:10-10:45 **PL-06:** T-cell immunoregulation in multiple sclerosis. Effect of C-phycocyanin and its combination with alpha Interferon. **Giselle Pentón (Cuba)**

10:45-11:15 **Break**

11:15-14:15 **Workshop and Symposium (Plaza America Convention Center, Varadero beach, Cuba)**

Revista Cubana de Farmacia vol. 42 (Suplemento 1):2, 2008

Sunday, April 20 **1st International Workshop of ImmunoPharmacology**

11:15-14:15 **ImmunoPharmacology Workshop.**

Chairs: Angus Dagleish (UK) and Ana Maria Hernandez (Cuba)

11:15-11:40 **L-01:** Activation of natural NEUGC-containing gangliosides related idiotypic networks in nsclc patients immunized with an anti-idiotype antibody. **Ana Maria Hernandez (Cuba)**

11:40-12:05 **L-02:** 1E10 anti-idiotype vaccine in non-small cell lung cancer. Experience in stage IIIB/IV patients. **Amparo Macias (Cuba)**

12:05-12:30 **L-03:** Treatment of high-grade glioma patients with the humanized anti-epidermal growth factor receptor (EGFR) monoclonal antibody NIMUTOZUMAB. **Patricia Marinello (Cuba)**

12:30-12:55 **L-04:** Relevance of monosyalyl lactosyl ceramides in cancer: their influence on tumor immunosurveillance. **Joel de León (Cuba)**

12:55-13:20 **L-05:** A novel mechanism in α ENaC regulation identified by molecular cloning, expression and co-expression of α EnaC alternatively spliced form "b" and α EnaC wildtype. **Marlene Shehata (Canada)**

13:20-13:45 **L-06:** Evaluation of the effects of *Zataria multiflora*, *Geranium pelargonium*, Myrth and Lemon essences on immune system function in experimental animals. **A. R. Khosravi (Iran)**

13:45-14:15 **L-07:** Immunosuppressive and phytochemical properties of *Tinospora bakis* Miers plant ethanolic extract. **Waleed Sayed Koko (Sudan)**

14:20 Return to Club Amigo Varadero Hotel (Lunch and continuation of others scientific and cultural activities).

Revista Cubana de Farmacia vol. 42 (Suplemento 1):3, 2008

Sunday, April 20 **5th International Workshop of Inflammation and Pain**

11:15-14:15 **Symposium on Inflammation: From disease mechanisms to novel drug targets**

Chairs: Andre G. Buret and John L. Wallace (Canada)

11:15-11:45 **L-08:** Biphasic role for lymphocytes in acute inflammation and its resolution. **Derek Gilroy (Canada)**

11:45-12:15 **L-09:** A therapeutic role for cannabinoids in the management of arthritis pain and inflammation. **Jason McDougall (Canada)**

12:15-12:45 **L-10:** H2S-releasing drugs: a new class of anti-inflammatories. **John L. Wallace (Canada)**

12:45-13:05 **L-11:** A Limulus- LALF32-51 derived peptide modulates the inflammatory response in macrophages exposed to lipopolisaccharide. **Maribel Guerra Vallespi (Cuba)**

13:05-13:30 **L-12:** A significant decrease of serum IL6 and MMP3 after etanercept treatment in patients with ankylosing spondylitis. **Chung-Tei Chou (Taiwan)**

13:30-13:55 **L-13:** Antinociceptive effects of guanosine in mice: Evidences for the mechanism of action. **Catiele Antunes (Brazil)**

13:55-14:15 **L-14:** Analgesic profile of Vimang tablets in patients with chronic pain. **Beatriz Garrido (Cuba)**

14:20 Return to Club Amigo Varadero Hotel (Lunch and continuation of others scientific and cultural activities)

Revista Cubana de Farmacia vol. 42 (Suplemento 1):4, 2008

Sunday, April 20 **1st International Workshop of NeuroImmunology**

11:15-14:15 **Symposium on Regulatory mechanism in NeuroImmunology**

Chairs: Carlos A Gonçalves (Brazil) and Alina González Quevedo (Cuba)

11:15-11:45 **L-15:** A putative role for brain markers. **Luis Portela (Brazil)**

11:45-12:05 **L-16:** Influence of melatonin on adult neurogenesis following global cerebral ischemia in Sprague-Dawley rats. **Moyosore Salihu Ajao (South Africa)**

12:05-12:25 **L-17:** Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat. **Évelin Vicente (Brazil)**

12:25-12:45 **L-18:** Evaluation of blood- cerebrospinal fluid barrier in Guillain-Barré Syndrome. **Alina González Quevedo (Cuba)**

12:45-13:05 **L-19:** Immune response in children with *Neisseria meningitidis* B meningoencephalitis. **Alberto Dorta Contreras (Cuba)**

13:05-13:25 **L-20:** Immuno-quantification of cytidines aimed at the diagnostic of neurodegenerative diseases. **Dannelys Pérez Bello (Cuba)**

13:25-13:55 **L-21:** Central nervous system derived LIGHT a decisive factor for recovery from experimental autoimmune encephalomyelitis. **Paula Mañá (Australia)**

13:55-14:15 **L-22:** Astroglial S100B secretion is stimulated by IL-1β: another piece in the "cytokine cycle" puzzle of Alzheimer's disease. **Carlos A. Gonçalves (Brazil)**

14:20 Return to Club Amigo Varadero Hotel (Lunch and continuation of others scientific and cultural activities)

Revista Cubana de Farmacia vol. 42 (Suplemento 1):5, 2008

Sunday, April 20

1st International Symposium about Pharmacology of Cytochrome P450

11:15-14:15 **Chairs: Rachel Tyndale (Canada) and Adrián Llerena (Spain)**

11:15-11:45 **L-23:** A genomic approach to the analysis of P450 regulation. **William Casley (Canada)**

11:45-11:15 **L-24:** Pharmacogenetic of drug metabolizing enzymes alters smoking cessation. **Rachel Tyndale (Canada)**

11:15-12:45 **L-25:** Effects and consequences of cytochrome P450 polymorphisms on the treatment of major depressive disorder. **Usoa Busto (Canada)**

12:45-13:15 **L-26:** Pharmacogenetic in Hispanic populations. **Adrián Llerena (Spain)**

13:15-13:45 **L-27:** CYP2C9 genetic polymorphism in a Cuban population. **Bárbaro Pérez (Cuba)**

13:45-14:15 **L-28:** Genetic polymorphism of cytochrome P450 2D6, 2C9, 2C19 and glycoprotein P in Cuban population. **Ioanna Martinez (Cuba)**

14:20 Return to Club Amigo Varadero Hotel (Lunch and continuation of others scientific and cultural activities).

14:30-16:00 **Lunch** (Club Amigo Varadero Hotel)

19:00-21:00 **Dinner** (Club Amigo Varadero Hotel)

21:00-22:00 **Poster Session** (Club Amigo Varadero Hotel):
ImmunoPharmacology

Chairs: Pedro Camilo Rodriguez, Eva Marrero, Gilberto Pardo, Grisel del Toro, Maria Acelia Marrero, Beatriz Garrido (Cuba)

Presentation of Special Issues of BLACPMA about "Natural Products and Drug Interactions". **Gabino Garrido (Cuba)**

PL-07: Modulation by Cuban natural health products on P450 enzymes: Potential interactions with therapeutic agents. **Idania Rodeiro (Cuba)**

22:00- **Music, dancing & karaoke**

Revista Cubana de Farmacia vol. 42 (Suplemento 1):6, 2008

Monday, April 21 **ImmunoPharmacology 2008**

At Plaza America Convention Center, Varadero beach, Cuba.

9:00-11:00 **Lectures.**

Chairs: Diogo Onofre Souza (Brazil) and Ariel Talavera (Cuba)

09:00-9:45 **PL-08:** Status for development of universal vaccines against meningococcal disease. **Johan Holst (Norway)**

9:45-10:30 **PL-09:** Proposal of a guanine-based purinergic system in the mammalian central nervous system. **Diogo Onofre Souza (Brazil)**

10:30-11:00 **PL-10:** Modeling the interaction between the anti- EFFR monoclonal antibody nimotuzumab and its target, TH epidermal growth factor receptor. **Ariel Talavera (Cuba)**

11:00-11:15 **Break**

11:15-14:15 **Workshop and Symposium (Plaza America Convention Center, Varadero beach, Cuba)**

Revista Cubana de Farmacia vol. 42 (Suplemento 1):7, 2008

Monday, April 21 **1st International Workshop of ImmunoPharmacology**

11:15-14:15 **Symposium on Vaccines**

Chairs: Coenraad Hendriksen (The Netherlands) and Mario Landys Chovel (Cuba)

11:15-11:40 **L-29:** Towards eliminating the use of animals for regulatory required vaccine quality control. **Coenraad Hendriksen (The Netherlands)**

11:40-12:05 **L-30:** The replacement of the *in vivo* assays for the quality control of human vaccines: utopia or reality? **Genevieve Waeterloos (Belgium)**

12:05-12:35 **L-31:** Vaccine potency assays: a Canadian regulatory perspective. **Aline Rinfret (Canada)**

12:35-13:00 **L-32:** Development of alternatives to pertussis vaccine control tests. **Dorothy Xing (UK)**

13:00-13:25 **L-33:** A new alternative for determining *in vitro* potency in vaccines containing Hepatitis B surface antigen. **Mario Landys Chovel (Cuba)**

13:25-13:50 **L-34:** Role of immunoepidemiology in the design of vaccination strategies. **Felipe Ochoa (Cuba)**

13:50-14:15 **L-35:** Mechanism of action of a GM3 ganglioside nanoparticulated vaccine in a preventive melanoma preclinical model. **Zaima Mazorra (Cuba)**

14:20 Return to Club Amigo Varadero Hotel (Lunch and continuation of others scientific and cultural activities)

Revista Cubana de Farmacia vol. 42 (Suplemento 1):8, 2008

Monday, April 21	**5th International Workshop of Inflammation and Pain**
11:15-14:15	**Symposium on strategies in pulmonary inflammatory processes.** **Chairs: Kim Abbenhaus (Novartis Pharma GmbH, Germany) and René Delgado (Cuba)**
11:15-11:45	**L-36:** Allergy and hypersensitivity: new insights and treatment options. **Randolf Brehler (Germany)**
11:45-12:15	**L-37:** Biotherapeutics as an opportunity for severe asthma. **Randolf Brehler (Germany)**
12:15-12:45	**L-38:** Modifiers of leukotrienes in the treatment of the asthma. **Roberto Águila de la Coba (Cuba)**
12:45-13:15	**L-39:** New immunological treatment approaches for allergic bronchopulmonary aspergillosis (ABPA). **Nicolas Schwerk (Germany)**
13:15-13:45	**L-40:** Recombinant interferon gamma in the treatment of life-threatening pulmonary diseases. **Idrián García (Cuba)**
13:45-14:15	**L-41:** Review on the anti-SARS works in hospitals in China. **Yan An (China)**
14:20	Return to Club Amigo Varadero Hotel (Lunch and continuation of others scientific and cultural activities)

Revista Cubana de Farmacia vol. 42 (Suplemento 1):9, 2008

Monday, April 21 **1st International Workshop of NeuroImmunology**

11:15-14:15 **Symposium on demyelinating diseases.**

Chairs: Hartmut Wekerle (Germany) and Maria A. Robinson (Cuba)

11:15-11:35 **L-42:** Studies of Cuprizone induced neuroinflammation. **David Linares Bandin (Australia)**

11:35-11:55 **L-43:** A demyelinating model induced by Cuprizone. Influence of transferrin and thyroid hormones. **Juana M. Pasquini (Argentina)**

11:55-12:15 **L-44:** The contribution of nitric oxide and interferon gamma to the regulation of the neuro-inflammation of experimental autoimmune encephalomyelitis. **David O. Willenborg (Australia)**

12:15-12:35 **L-45:** Repeated pro-inflammatory demyelinating stimuli may result in reduced inflammation and demyelination. **Verónica Murta (Argentina)**

12:35-12:55 **L-46:** Neuroimmunoregulation, a tool for therapy in demyelinating diseases. **María A. Robinson (Cuba)**

12:55-13:15 **L-47:** T cell immunoregulation and oxidative stress in neuromielytis optica. **Majel Cervantes (Cuba)**

13:15-13:35 **L-48:** Therapeutic interventions in the treatment of multiple sclerosis. **Jean A. Merrill (USA)**

13:35-13:55 **L-49:** Multiple sclerosis in childhood: Scientific evidences. **Hector Vera (Cuba)**

13:55-14:15 **L-50:** Neuromyelitis optica spectrum disorders in Cuba. **Jose A. Cabrera-Gómez (Cuba)**

14:20 Return to Club Amigo Hotel (Lunch and continuation of others scientific and cultural activities)

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Monday, April 21

1st International Symposium about Pharmacology of Cytochrome P450

Chair: Micheline Piquette Miller (Canada) and Javier Espinosa (Mexico)

11:15-11:45 **L-51:** Role of cytochrome P450 modulation on mutagen activation and drug-drug interactions. **Javier Espinosa (México)**

11:45-12:15 **L-52:** Regulation of drug transporters in health and disease. **Micheline Piquette Miller (Canada)**

12:15-12:45 **L-53:** Impact of inflammation on drug clearance pathways in cancer. **Graham Robertson (Canada)**

12:45-13:15 **L-54:** Cytochromes P450 and non-steroidal anti-inflammatory drugs, prevention of gastrointestinal bleeding. **José Agundez (Spain)**

13:15-13:45 **L-55:** From pharmacogenetic to personalized medicine: a regulatory perspective. **Diadelis Remirez (Cuba)**

13:45-14:15 **General discussion**

14:20 Return to Club Amigo Varadero Hotel (Lunch and continuation of others scientific and cultural activities)

14:30-15:30 **Lunch** (Club Amigo Varadero Hotel)

19:00-21:00 **Dinner** (Club Amigo Varadero Hotel)

21:00-22:00 **Poster Session** (Club Amigo Varadero Hotel):

Inflammation, Pain and Cytochrome P450

Chairs: Pedro Camilo Rodriguez, Eva Marrero, Gilberto Pardo, Grisel del Toro, Maria Acelia Marrero, Beatriz Garrido (Cuba)

NeuroImmunology

Chairs: Jean A Merrill (USA), Nancy Pavón (Cuba)

PL-11: Immunotoxicity evaluation in safety assessment of drugs. Current and future perspectives. **Alexander Batista (Cuba)**

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Presentation of the leaders of Cuban Scientific Institutions in the field of ImmunoPharmacology:

- Silvio Perea, Center for Genetic Engineering and Biotechnology (CIGB)
- Rolando Perez, Centre of Molecular Immunology (CIM)
- Mario L. Chovel, Finlay Institute
- Alexander Batista, Centre of Toxicology and Biomedicine (TOXIMED)
- René Delgado, Center of Pharmaceutical Chemistry (CQF)
- José A. Cabrera-Gómez, International Center of Neurological Restoration (CIREN)

22:00-23:50 **Farewell party (**Club Amigo Varadero Hotel)

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Tuesday, April 22 **ImmunoPharmacology 2008**

At Plaza America Convention Center, Varadero beach, Cuba.

10:00-12:00 **Lectures**

Chairs: Rolando Pérez and Silvio Perea (Cuba)

10:00-10:30 **PL- 12:** Mechanisms of immune mediated injury and repair in the Central Nervous System. **Jack Antel (Canada)**

10:30-11:00 **PL- 13:** Biopharmaceutical approach to autoimmune mechanisms in neurological disease therapy. **Eduardo Pentón - Arias (Cuba)**

11:00-11:30 **PL- 14:** Protein kinases as promising pharmacologic targets for cancer therapeutics. Developing of CIGB-300, a novel proapoptotic synthetic peptide that impairs the protein kinase CK2-mediated phosphorylation. **Silvio Perea (Cuba)**

11:30-12:00 **PL- 15:** Ganglioside-based cancer immunotherapy: Two vaccination approaches. **Rolando Pérez (Cuba)**

12:00-12:30 **Break**

12:30-13:00 **Closing lecture**

PL-16: Immunopharmacological researches in Cuba. Results and perspectives. Its impact in Cuban system of health. **René Delgado (Cuba)**

13:00-14:15 **Closing Ceremony**

Report of the Congress. **Emilio Monteagudo (Cuba)**

Awards of the "ImmunoPharmacology 2008" **Gabino Garrido (Cuba)**

Announce of Next Congresses of the Cuban Society of Pharmacology. **Evangelina Marrero Faz (Cuba)**

14:30-15:30 **Lunch** (Club Amigo Varadero Hotel)

16:30 Return the delegates to Havana and others provinces

Revista Cubana de Farmacia vol. 42 (Suplemento 1):13, 2008

Posters

Sunday, April 20 **Posters of ImmunoPharmacology (PIP)**

Chairs: Pedro Camilo Rodriguez, Eva Marrero, Gilberto Pardo, Grisel del Toro, Maria Acelia Marrero, Beatriz Garrido (Cuba)

PIP-01 CHARACTERIZATION OF A NON-APOPTOTIC CELL DEATH INDUCED BY AN ANTI-N-GLYCOLYL GM3 ANTIBODY. **Roque-Navarro L, Chakrabandhu K, de León J, Rodríguez S, Toledo C, Carr A, Mateo de Acosta C, Hueber AO and Pérez R (Cuba)**

PIP-02 ALTERATIONS OF TH1, TH2 AND TH3 POLARIZATION IN MAJOR DEPRESSION: EFFECT OF SERTRALINE THERAPY. **Oktenli C, Sutcigil L, Musabak U, Cansever A, Uzun O, Sanisoglu SY, Bozkurt A, Yesilova Z, Ozmenler N, Ozsahin A, Sengul A (Turkey)**

PIP-03 INDUCTION OF TRANSPLANTATION TOLERANCE BY ALLOGENEIC DONOR-DERIVED CD4+CD25+FOXP3+ REGULATORY T CELLS. **Velásquez-Lopera M, Eaton Valerie, Lerret N, Correa L, DeCresce R, García LF, Jaramillo A**

PIP-04 T CELLS ARE CRUCIAL FOR THE ANTI-METASTATIC EFFECT OF ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR ANTIBODIES. **Garrido G, Rabasa R, Lorenzano P, Sanchez B, Irene Beausoleil, López A, Alonso DF, Pérez R, Fernández LE (Cuba)**

PIP-05 SPECIFIC IMMUNE RESPONSE INDUCED BY IMMUNIZATION WITH THE AUTOLOGOUS EPIDERMAL GROWTH FACTOR RECEPTOR-EXTRACELLULAR DOMAIN. **Sánchez Ramírez B, Suárez Pestana E, Aguiar Y, Garrido Hidalgo G, Hernández DR, Pérez Rodríguez R, Ullrich A and Férnandez LE (Cuba)**

PIP-06 PHARMACOLOGIC EVALUATION OF A NOVEL PROAPOPTOTIC PEPTIDE THAT IMPAIRS THE PROTEIN KINASE (CK2) PHOSPHORYLATION IN TUMOR ANIMAL MODELS. **Perera Y, Farina E, Hernandez I, Mendoza O, Serrano JM, Reyes O, Bacardi D, Alba J, Vazquez A, Cosme K, Gomez DE, Gomez RE, Acevedo BE, Alonso DF, Perea SE (Cuba)**

PIP-07 ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR ANTIBODIES DECREASE TUMOR GROWTH PROMOTED BY RADIATION THERAPY. **Díaz A, Rolff J, Lemm M, Fichtner I, and Montero E (Cuba)**

PIP-08 ASSESSMENT OF CANDIDATE IMMUNOPOTENTIATOR CM-95 SOLUTION UNDER MAGNETIC TREATMENT OVER PARAMETER BLOODY-RED AND TISSUE OF BALB/C MOUSE. **Martínez CE, Castillo JR, Favier P, Tamayo V, Pardo AM, Sierra VG (Cuba)**

PIP-09 REDUCTION OF ACETAMINOPHEN-INDUCED HEPATOTOXICITY BY PRE-TREATMENT WITH FREUND´S ADJUVANTS IN MICE. **Batista AD, Pérez N, Fong O, Betancourt J, Salas H; Portuondo D (Cuba)**

PIP-10 WOUND HEALING IN PRECLINICAL PHARMACOLOGY. INFLUENCE OF THE EXPERIMENTAL DESIGN OVER RAT IMMUNE SYSTEM MAIN ORGANS MORPHOLOGY. **Merino N, Subiron N, Oruña L, Luque Y (Cuba)**

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PIP-11 POTENTIAL BIOCHEMICAL SERUM MARKERS OF VASCULAR INJURY IN CHRONIC CYCLOSPORINE TREATED RATS. **Böhmer AE, Brum LMBP, Souza DG, Oses JP, Viola GG, Silva VD, Lopes TG, Bruch RS, Sarkis JJF, Portela LV, Souza DO (Brazil)**

PIP-12 EVALUATION OF THE *in vitro* VERO CELL ASSAY AS ALTERNATIVE TO THE *in vivo* TOXIN NEUTRALIZATION TEST FOR DIPHTHERIA VACCINES POTENCY. **Lara A, García M, Rodríguez O, Remírez D (Cuba)**

PIP-13 EVALUATION OF ANTIPROLIFERATIVE EFFECT OF THE IFNA/C-PHYCOCIANIN COMBINATION IN HEP-2 TUMOR CELLS. **López Ocejo O, Pentón Rol G, Magariño Fariña J and Delgado Hernández R (Cuba)**

PIP-14 EFFECT OF *Mangifera indica* L EXTRACT (VIMANG) ON HUMAN LUNG (H460) AND COLON (HT-29) TUMOR CELLS PROLIFERATION. **Pardo Andreu GL, de Farias CB, Maurman N, Delgado R, Roesler R (Cuba)**

PIP-15 *In vitro* STUDY OF IMMUNOMODULATORY ACTIVITY OF AQUEOUS INFUSION OF *Bidens pilosa.* **Boffill M, del Campo J, Méndez M, Abajo C, González Y, Montserrat Mitjans M, Vinardell MP (Cuba)**

PIP-16 *In vitro* LEISHMANICIDAL ACTIVITY OF ELEVEN ARTEMISIA SPECIES OF IRAN. **Zamani TR Sh, Mahmoudi M, Emami A, Ahi A, Siadat Z (Iran)**

PIP-17 THE CYTOTOXIC EFFECTS OF SOME FRACTIONS ISOLATED FROM *Pleurotus florida* BODY EXTRACT ON CANCER CELL LINES. **Mahmoudi M, Ghazanfari T, Zamani TR Sh, Yaraee R, Siadat Z (Iran)**

PIP-18 STUDY THE CYTOTOXICITIC AND PRO-APOPTOTIC EFFECTS OF *Pleurotus florida* BODY EXTRACT ON CANCER CELL LINES. **Ghazanfari T, Mahmoudi M, Zamani TR Sh, Yaraee R, Siadat Z (Iran)**

PIP-19 IMMUNE RESPONSE IN MICE BALB/C TO RECOMBINANT *Streptomyces lividans* SECRETING *Mycobacterium tuberculosis* APA AND THE WILD TYPE STRAIN AS A LIVE VECTOR FOR VACCINE PREPARATIONS AGAINST TB. **Vallín C, Ayala JC, García D, Jones J, Rodríguez C, Hernández I, Pimienta E, Gonzalez L, Vila A, Olivares N, Sarmiento ME, Acosta A, Van Mellaert L, and Anné J (Cuba)**

PIP-20 THE USE OF STREPTOMYCES FOR IMMUNIZATION AGAINST MYCOBACTERIAL INFECTIONS. **Olivares Arzuaga N, Vila Granda A, Ramírez JC, Marquez Domínguez J, Sarmiento García San Miguel ME, Vallín Plous CR, Rodríguez Valdés C, Infante Bourzac JF, Ramos Morí A, González Mesa L, López Hernández Y, Acosta Domínguez A (Cuba)**

PIP-21 SYNERGISTIC STIMULATION OF PROLIFERATION OF U138-MG GLIOBLASTOMA CELLS BY GASTRIN-RELEASING PEPTIDE IN COMBINATION WITH AGENTS THAT ENHANCE CAMP SIGNALING. **de Farias CB, Lima RC, Lima LO, Flores DG, Meurer L, Brunetto AL, Schwartsmann G, Roesler R (Brazil)**

PIP-22 ROLE OF AMNIOTIC MEMBRANE AS BIOLOGICAL CURATIVE ON SURGICALLY DAMAGED LIVER, UNDER THE INFLUENCE OF VERAPAMIL, IN RATS. **Vilela-Goulart MG, Gomes MF, Salgado MAC, Oliveira MAC, Bastos-Ramos WP (Brazil)**

PIP-23 FUNCTIONAL REGENERATION OF THE SURGICALLY DAMAGED LIVER AFTER TREATMENT WITH AMNIOTIC MEMBRANE AND VERAPAMIL, IN RATS. **Vilela-Goulart MG,**

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Gomes MF, Salgado MAC, Valva NV, Bastos-Ramos WP (Brazil)

PIP-24 EVALUATION OF THE EXPERIMENTAL REDUCTION AND REMOVING OF THIOMERSAL OVER THE EFFICACY AND SAFETY OF VACCINES FOR HUMAN USE. **Avilés A, Chovel ML (Cuba)**

PIP-25 CHARACTERIZATION OF REFERENCE MATERIALS FOR THE IMPLEMENTATION OF AN ELISA FOR DETERMINING ANTI-TETANUS ANTIBODIES IN GUINEA- PIG SERUM. **Ontivero I, Ochoa R, Lara M, Landys Chovel M, Mahy T, Cisneros D, Brenda Serrano, Cruces A, Gutierrez N, Hernández P, Barrios T, Herrera L (Cuba)**

PIP-26 COMPARED STABILITY STUDY OF A TETANUS VACCINE WITH TRADITIONAL AND REDUCED ANTIGEN CONTENT. IMPACT OVER THE BIOLOGICAL ACTIVITY. **Mandiarote A, Pérez E, Sosa R, Labrador I, Muñoz Y, Solano S (Cuba)**

PIP-27 CHARACTERIZATION OF WORKING REFERENCE STANDARDS FOR DETERMINING THE POTENCY OF TETANUS VACCINES ACCORDING TO WHO APPROACH. **Mahy T, Bourg V, Cisneros D, Noroña M, Ontivero I, Morejon A, Herrera L, Gutiérrez N, Alpizar J, Huergo L (Cuba)**

PIP-28 METHODOLOGY FOR THE FORECAST OF THE IMMUNOGENICITY / AUTOIMMUNITY OF EXOGENS PROTEINS. **Barreiro AV, Ramirez K (Cuba)**

PIP-29 PREDICTION OF MOLECULAR MIMETISM BETWEEN RECOMBINANT STREPTOQUINASA AND HUMAN PROTEINS. **Ramírez K, Barreiro AV (Cuba)**

PIP-30 REGULATORY IMMUNE RESPONSES INDUCED BY AN APL FROM HEAT-SHOCK PROTEIN 60. **Barberá A, Domínguez MC, Lorenzo N, Torres LE, Almaguer M, Torres AM, Hernández MV, Darrasse-Jèze G, Klatzmann D and Padrón G (Cuba)**

PIP-31 PHARMACOKINETIC ALTERNATIVES APPLIED TO THE ESTABLISHMENT OF THE BIOLOGICAL OPTIMAL DOSING (BOD): EXPERIENCE WITH AND MONOCLONAL ANTIBODY ANTI EGF-R. **Fernández Sánchez E (Cuba)**

PIP-32 SAFETY AND PHARMACOKINETIC EVALUATION OF ANTI EGF RECEPTOR HUMANIZED MONOCLONAL ANTIBODY NIMOTUZUMAB (HR3) IN LOCALLY ADVANCED BREAST CANCER TUMOURS IN THE NEOADYUVANT SETTING IN COMBINATION WITH CHEMOTHERAPY REGIMEN. PHASE I CLINICAL TRIALS PRELIMINARY RESULTS. **Ramos-Suzarte M, Soriano JL, Batista N, Lima M, Rodriguez R, Gonzalez J, Rodriguez-Vera L, Leonard-Rupale I, Montenegro A, Fernandez E, Garcia R, Suárez N, Viada CE, Crombet-Ramos T (Cuba)**

PIP-33 VALIDATION OF TWO IMMUNOASSAYS FOR HAMA AND PK STUDIES IN CLINICAL TRIALS WITH NIMOTUZUMAB THERAPY IN CANCER PATIENTS. **Rodríguez-Vera L, Leonard-Rupalé I, Crombet-Ramos T and Ramos-Suzarte M (Cuba)**

PIP-34 OVER EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN ANAL CANAL CARCINOMA AFTER TREATMENT WITH RADIOTHERAPY. **Llorente FF. Rengifo E. Ramos M. Rengifo ChE. Cedeño M. Blanco R. Frómeta M (Cuba)**

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PIP-35 PRIMING AND BOOSTING DETERMINANTS ON THE ANTIBODY RESPONSE TO AN EGF-BASED CANCER VACCINE. **Rodríguez PC, González I, Montero E and Lage A (Cuba)**

PIP-36 IMMUNOCHEMOTHERAPY WITH ANTI-CD20 MONOCLONAL ANTIBODY RITUXIMAB IN THE TREATMENT OF RELAPSED INDOLENT LYMPHOMA. **Lami L, Areces F, Vásquez E , Rodríguez A , Díaz C, Mederos N (Cuba)**

PIP-37 EXPERIENCE WITH ADVERSE EVENTS ASSOCIATED WITH ACM H-R3 (THERACIM®). **Aguilera N, Neninger E, Hernández A, Macías A, Alfonso K (Cuba)**

PIP-38 PRESENCE OF TITLES ANTI-INTERFERON ANTIBODIES A PROGNOSIS FACTOR OF THE CLINICAL RESPONSE RECOMBINAT INTERFERON. **Pérez M, Cid M, Arbolaéz M, Méndez R, Rodríguez M (Cuba)**

PIP-39 ADJUVANT INTERFERON GAMMA IN PATIENTS WITH PULMONARY ATYPICAL MICOBACTERIOSIS: A RANDOMIZED CONTROLLED STUDY. **García-García I, Milanés-Mirelles MT, Santos-Herrera Y, Valdés-Quintana M, Valenzuela-Silva C, Jiménez-Madrigal G, Ramos-Gómez T, Bello-Rivero I, Fernández-Olivera N, Suárez-Méndez R, Carbonell-Freire D, González-Méndez L, Martínez-Sánchez G, López-Saura P, for the MACGAM Study Group. (Cuba)**

PIP-40 PHARMACOKINETICS ALTERNATIVE FOR THE EVALUATION OF IFN A-2B PEYILATE MOLECULES. **Fernández RL (Cuba)**

PIP-41 ADYUVANT THERAPY WITH RECOMBINANT INTERFERON-ALPHA 2B IN THE TREATMENT OF PATIENTS WITH HIGH RISK MELANOMA. **Ruíz Calabuch H, Ramos L, Romero P, Oviedo M, Marín J, León O, Benítez I (Cuba)**

PIP-42 USE OF RECOMBINANT HUMAN INTERFERON-ALPHA 2B IN HIGH-RISK MELANOMA PATIENTS. **Alfonso K, González L, Ávila Y, Anasagasti L, Ruiz H, Valenzuela C, Ballagas C (Cuba)**

PIP-43 HASHIMOTO'S AUTOIMMUNE THYROIDITIS. RESPONSE TO IMMUNOSUPRESSOR THERAPY USING GLYCOCORTICOIDS. **Valdés R, Barrios R, Trabanco M (Cuba)**

PIP-44 GRAVE'S OPHTHALMOPATHY AND THE USE OF THE IMMUNOMODULATOR CYCLOPHOSPHAMIDE (ENDOXAN): OUR EXPERIENCE. **Valdes R, Trasanco M (Cuba)**

PIP-45 CASE REPORT: USE OF THE HEBERBIOVAC-HB VACCINE AND HYPERIMMUNE GAMMA GLOBULIN IN AN INFANT BORN FROM A HBSAG-CARRIER MOTHER. **Acosta J, González A, Caro R, González V, Dacourt A, García E, Martínez A (Cuba)**

PIP-46 CELL AND HUMORAL IMMUNE RESPONSE IN IMMUNE DEPRESSED VACCINED PATIENTS WITH THE B ANTI HEPATITIS CUBAN VACCINE. **Jauma Rojo AJ, Insua Arregui C, Macias Abraham C, Gonzalez Labrada C, Bericiartu M (Cuba)**

PIP-47 EVALUATION OF THE EFFECTIVENESS OF THE *Aloe barbadensis* EXTRACT FOR INYECTION IN INMUNO DEFICIENT PATIENTS. **Rodríguez Acosta M, Castellanos Puerto E, Sin Mayor A, Moya A (Cuba)**

PIP-48 EFFECTIVENESS OF INTACGL0BIN IN PATIENTS WITH IDIOPATIC THROMBOCYTHOPENIC PURPURA PREVIOUSLY TO TOTAL SPLENECTOMY. **Prado Vizcaino Y, García Peralta T, Castillo González D, Almagro Vázquez D, Prado Vizcaino E (Cuba)**

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PIP-49 THE IMMUNOGLOBULIN IGG THROUGH INTRAVENOUS ADMINISTRATION AS AN ADJUVANT THERAPY IN THE SEPSIS OF ELDER PATIENTS WITH ACUTE LEUKAEMIA. **Cabrera M, Fernández JA, Guerra T, Tioples S, Rodríguez D (Cuba)**

PIP-50 IMMUNOSENESCENCE: FIRST MEASUREMENTS IN HEALTHY CUBANS. **García B, Badía T, Leonard I, Huerta P, Tait C, Fonseca EE, Travieso G, Mazorra Z, Lage A (Cuba)**

PIP-51 IMMUNOTHERAPY IN BREAST, COLON AND OESOPHAGUS CANCER: MANAGEMENT OF CLINICAL TRIALS IN SANTIAGO DE CUBA. **Griñán D, Perdomo D, Landazuri S, Alvarez R, Peacok S, Saumell Y, Ortiz L (Cuba)**

PIP-52 USE OF IMMUNOTHERAPEUTIC PRODUCTS IN CLINICAL ASSAYS PERFORMED IN SANTIAGO DE CUBA. AN OVERVIEW. **Perdomo D, Griñán D, Landazuri S, Alvarez R, Peacok S (Cuba)**

PIP-53 THE IMMUNOGENICITY AND REACTOGENICITY OF THE QUIMIOHIB CUBAN VACCIN IN UNDER ONE- YEAR-OLD HEALTHY INFANTS. **Gavilla González BC, Alonso Gutierrez MF (Cuba)**

PIP-54 IMMUNOMODULATOR PRESCRIPTIONS IN NEONATES WITH SEPSIS AT THE NORTH PEDIATRIC HOSPITAL OF SANTIAGO DE CUBA. **Delgado B, Ramírez E, Kindelán L, Pérez L (Cuba)**

PIP-55 ABDOMINAL INFECTION, IMMUNE RESPONSE. REVIEW OF THE BIOLOGICAL, MOLECULAR, PHYSIOLOGICAL, AND CLINICAL ALTERATIONS. **Marrero Miragaya MA, Araña Rosainz MJ, Pastrana Román I (Cuba)**

PIP-56 IMMUNITY AND FERTILITY - LIF GENE MUTATIONS IN WOMEN DIAGNOSED WITH UNEXPLAINED INFERTILITY AND ENDOMETRIOSIS HAVE NEGATIVE IMPACT ON THE IVF OUTCOME. **Rokyta Z, Novotny Z, Sima R, Kralickova M (Czech Republic)**

PIP-57 PRESCRIPTION OF THE CUBAN MONOCLONAL ANTIBODY IOR-T3 IN PATIENTS WITH STEROID-RESISTANT ACUTE REJECTION IN KIDNEY TANSPLANTATION. **Tamayo J, Morales M, Lambert J, Omar Z (Cuba)**

PIP-58 EVEROLIMUS, AN APPROACH TO ITS EFFICIENCY IN RENAL TRASPLANT. **Lara C, Cires M (Cuba)**

PIP-59 IMMUNE MECHANISM OF THE PRINCIPLES HIPERSENSITIVITY ADVERSE DRUG REACTIONS REPORTED TO THE CUBAN SURVEILLANCE SYSTEM. **Calvo D, Jiménez G (Cuba)**

PIP-60 HYPERSENSITIVITY REACTIONS ARE FREQUENT IN CUBA? DATA FROM THE DRUG SURVEILLANCE CUBAN PROGRAMME. **Jiménez G, Calvo D (Cuba)**

PIP-61 DETECTION OF POSITIVE SELECTION IN COMPLETE GENOMES OF HUMAN PATHOGENS. **Martínez-Pérez O, Pajón Feyt R and Carrasco-Velar R (Cuba)**

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Monday, April 21 **Posters of Inflammation & Pain (PIF)**

Chairs: Pedro Camilo Rodriguez, Eva Marrero, Gilberto Pardo, Grisel del Toro, Maria Acelia Marrero, Beatriz Garrido (Cuba)

PIF-01 ROLE OF iNOS-DERIVED NITRIC OXIDE IN INDOMETHACIN-INDUCED INTESTINAL DAMAGE. **Riaño A, Ortiz-Masià D, Hernández C, Paniagua M, Esplugues JV y Barrachina MD (Cuba)**

PIF-02 THE EFFECT OF iNOS INHIBITORS TREATMENT IN EXPERIMENTAL COLITIS MODELS. **Yesilova Z, Ercin CN, Korkmaz A, Ozcan A, Uygun A, Dagalp K (Turkey)**

PIF-03 ANTIINFLAMMATORY EFFECTS OF SYNTHETIC 1-O-NONANYLGLYCEROL IN ADYUVANT-INDUCED CHRONIC INFLAMMATION MODE. **Pech W, Del Toro G, Becquer MA, Fraga A, León JL, Valdés Y (Cuba)**

PIF-04 SYNTHETIC 1-O-UNDECYLGLYCEROL:*in vivo* ANTIINFLAMMATORY ACTION. **Del Toro G, Pech W, Becquer MA, Fraga A, León JL, Valdés Y, Trapero YM (Cuba)**

PIF-05 EFFECTS OF MELATONIN, FISH OIL AND VITAMIN E ON THE CYCLOOXYGENASE-2 ACTIVITY AND OXIDATIVE STRESS IN MIDDLE BRAIN OF C57/BL6 MICE AFTER THE ADMINISTRATION OF 1-METHYL-4-PHENYL-1, 2,3,6 - TETRAHYDROPYRIDINE (MPTP). **Ortiz GG, Pacheco-Moisés FP, Gómez V, Barba EA, Sánchez-González VJ, González RE and Velázquez-Brizuela IE (Mexico)**

PIF-06 SESQUITERPENE LACTONE FRACTION FROM *Artemisia khorassanica* INHIBITS INOS AND COX-II EXPRESSION THROUGH THE INACTIVATION OF NF-KB. **Zamani TR Sh, Mahmoudi M, Emami A, Iranshahi M, Siadat Z (Iran)**

PIF-07 INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION BY THE KOPETDAGHINS FROM *Dorema kopetdaghense* IN J774A.1 MACROPHAGES. **Zamani TR Sh, Mahmoudi M, Ghazanfari T, Iranshahi M, Siadat Z (Iran)**

PIF-08 ANTINFLAMMATORY EFFECTS EVALUATION OF A CARBOHYDRATE OF *Bromelia pinguin* USING THREE NON-CLINICAL MODELS. **Monteagudo E., Monteagudo G, Boffill M, Díaz LE, Verdecía B (Cuba)**

PIF-09 BONE PROTECTIVE EFFECTS OF D-003 IN EXPERIMENTAL MODELS OF PRIMARY AND SECONDARY OSTEOPOROSIS. **Noa M, Mendoza S, Más R and Mendoza N (Cuba)**

PIF-10 PRECLINICAL VALUTATION OF THE ANTIINFLAMMATORY ACTIVITY OF WHITE BIDENS (ROMERILLO). **Martínez Novellas Y (Cuba)**

PIF-11 ANTI-INFLAMMATORY EFFECTS OF *Musa paradisiaca* L EXTRACT (ACITAN®). **Pérez MR, Rodríguez CC, Martínez G, Horta D (Cuba)**

PIF-12 ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF EXTRACTS FROM THE STEM BARK OF *Croton macrostachyus* (EUPHORBIACEAE) IN RATS AND MICE. **Mbiantcha M, Nguelefack TB, Watcho P, Ndontsa BL, Ateufack G, Pierre T, Kamanyi A (Cameroon)**

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PIF-13 EFFECT OF GENDER ON THE ANGIOGENESIS AND INFLAMMATORY PARAMETERS IN THE RAT AIR POUCH MODEL OF INFLAMMATION. **Eteraf Oskouei T, Maleki-Dizaji N, Garebageri A, Najafi M (Iran)**

PIF-14 POTENTIALITIES IN ANTIINFLAMMATORY THERAPY IN THE ALGAE *Galaxaura rugosa* AND *Dichotomaria obtusata*. **Dutok CM, Frías AI, Vidal A, García N, Carnesoltas D, López T (Cuba)**

PIF-15 IL-1β FURTHER ATTENUATES DIMINISHED ANALGESIC EFFECT OF MORPHINE IN DIABETIC MICE. **Yildiz O, Gul H, Dogrul A, Yesilyurt O (Turkey)**

PIF-16 EFFECTS OF *Cynodon dactylon* (L.) pers. ON CARDIAC HEMODYNAMIC FUNCTIONS DURING ISCHEMIA AND REPERFUSION. **Najafi M, Nazemiyeh H, Ghavimi H, Gharakhani A, Garjani A (Iran)**

PIF-17 EFFECT OF PERINEURAL BETAMETHASONE ON NERVE COMPRESSION DURING SPINAL COLUMN SURGERY. **Bárzaga Z, Puente A, Lopez A (Cuba)**

PIF-18 ANTIINFLAMMATORY EFFECTS OF MULTIPLE DOSES OF A DICLOFENAC-LYSINE CLONIXINATE COMBINATION IN RATS. **Pérez-Urizar J, Torres-Roque I, Aguilera-Suárez G, Gómez-Sánchez M (Mexico)**

PIF-19 PHARMOCOKINETICS OF DICLOFENAC-LYSINE CLONIXINATE COMBINATION IN HEALTHY VOLUNTEERS. **Torres-Roque I, Pérez-Urizar J, Pérez-Flores G, Viruete-Cisneros S, Galaviz-Muro A, Morales M, Aguilera-Suárez G, Gómez-Sánchez M (Mexico)**

PIF-20 HANDLING OF PAIN IN PATIENTS WITH TERMINAL CANCER, TREATED WITH MORPHINE. **Bermudez I, Cereijo D (Cuba)**

PIF-21 ANALGESIC PHARMACOTHERAPY ON CANCER PATIENTS ON TERMINAL STAGE. **Real N, Gainza S, Lores D (Cuba)**

PIF-22 APPLICATION OF THE MAGNETIC INDUCTIVE STABILIZER (EIMA®) IN THE TREATMENT OF PATIENTS WITH RHEUMATOID ARTHRITIS. **Yepes A, Aguilera E, Gómez R, Aguilar B, Kindelán L (Cuba)**

PIF-23 EFFECT OF THE TREATMENT WITH MONTELUKAST IN PATIENTS ASTHMATICS. **Aguila de la Coba R, Castillo Mendez A (Cuba)**

PIF-24 TREATMENT OF ATOPIC DERMATITIS WITH AN AQUEOUS EXTRACT OF *Mangifera indica* L. (VIMANG®). **Guevara M, Pérez T, Perdomo, Morales C, Garrido G (Cuba)**

PIF-25 OXIDATIVE STRESS AND ADVERSE OUTCOMES IN PATIENTS WITH CORONARY ARTERY DISEASE. **Mitrovska S, Jovanova S (Macedonia)**

PIF-26 SYSTEMIC INFLAMMATORY RESPONSE: PHYSIOPATHOLOGY AND MEDIATORS. **Santeliz J, Martínez J, Peña Y, Ochoa O (Venezuela)**

PIF-27 ELECTRONIC BOOK: INFLAMMATION AND PAIN. ANSWER OF INMUNULOGYCAL SYSTEM AND NEW THERAPEUTIC STRATEGIES. **Pereira E, Cardosa E, Dorado L, Rivera N, Fernández Y, Trujillo R (Cuba)**

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Monday, April 21 **Posters of Cytochrome P450 (PC)**

Chairs: Pedro Camilo Rodriguez, Eva Marrero, Gilberto Pardo, Grisel del Toro, Maria Acelia Marrero, Beatriz Garrido (Cuba)

PC-01 GLYCOPROTEIN P GENETIC POLYMORPHISM IN A REPRESENTATIVE SAMPLE OF CUBAN POPULATION. **Martinez I, Kirchheiner J., Rodeiro I, Álvarez M; Pérez B and Rodríguez Y (Cuba)**

PC-02 DEBRISOQUINE POLYMORPHISM IN A SAMPLE OF CUBAN POPULATION. **Álvarez M, Pérez B, Llerena A, Labacena M, García L, Rojo D (Cuba)**

PC-03 INHIBITION OF HUMAN P450 ENZYMES BY NATURAL EXTRACTS USED IN TRADITIONAL MEDICINE. **Rodeiro I, Donato MT, Jiménez N, Garrido G, Molina-Torres J, Menéndez R, Castell JV and Gómez-Lechón MJ (Cuba)**

PC-04 INTERACTIONS ON CYTOCHROME P450 DRUGS METABOLISM, IN HOSPITALIZED PATIENTS WITH NEUROLOGICAL DISEASES. **Dupotey NM, Pupo M, Matos K, Fernández Y, Ramos B, Veranes R (Cuba)**

PC-05 RESPONSE TO ANTIDEPRESSIVE DRUGS DURING ANTIVIRAL THERAPY IN CHRONIC HEPATITIS C CUBAN PATIENTS. **Sánchez Y (Cuba)**

PC-06 TENDENCIES IN THE INVESTIGATIONS AND PUBLICATIONS IN HERBAL-DRUG INTERACTIONS. **Valdés M, Garrido G (Cuba)**

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Monday, April 21 **Posters of NeuroImmunology (PNI)**

Chairs: Jean A Merrill (USA), Nancy Pavón (Cuba)

PNI-01 BONE MARROW CELLS TRANSPLANT ON THE MOTORS ASYMMETRIES AND THE MEMORY-LEARNING DYSFUNCTIONS IN RATS WITH STRIATAL LESION WITH QUINOLINIC ACID. **Serrano Sánchez T, Martínez Martí L, García Minet R, Alberti Amador E, Blanco Lezcano L, Castillo Díaz L, Lorigados Pedre L, Pavón Fuentes N, Fernandez Verdecia I (Cuba)**

PNI-02 BIOCHEMICAL AND BEHAVIORAL EFFECT OF GLUTATHIONE DEPLETION IN RAT BRAIN. **González Fraguela ME, Blanco Lezcano L, García Miniet R, Lorigados Pedre L, Bauza Calderin JY (Cuba)**

PNI-03 DOPAMINE EFFECTS ON STRIATAL MITOCHONDRIAL FUNCTION. **Czerniczyniec A, Bustamante J, Lores Arnaiz S (Argentina)**

PNI-04 EFFECTS OF THE MK-801 TREATMENT ON THE AMINOACID NEUROTRANSMITTER RELEASE AND CELL DEATH PROCESS IN PEDUNCULOPONTINE NUCLEUS OF HEMIPARKINSONIAN RATS. **Blanco Lezcano L, Lorigados Pedre L, González Fraguela ME, Martínez Martí L, Pavón Fuentes N, Bauzá Y (Cuba)**

PNI-05 TRANSPLANTATION OF STROMAL CELLS SUSPENSIONS IN STRIATUM NUCLEUS IN THE RAT MODEL OF PARKINSON'S DISEASE. **Pavón-Fuentes N, Blanco-Lezcano L, Martínez-Martín L, Castillo-Díaz L, Cuétara-Bernal K, García-Miniet R, Lorigados-Pedre L, Coro-Grave de Peralta Y, García A, Macías-González R (Cuba)**

PNI-06 EVALUATION OF THE IMMUNOLOGIC CHANGES IN THE TEMPORAL LOBE EPILEPSY BEFORE AND AFTER OF THE LOBECTOMY. **Lorigados L, Pavon N, Serrano T, Robinson MA, Morales L, Garcia I, Bender JE, Macias R, Galbizu R, Bauza Y (Cuba)**

PNI-07 CHARACTERIZATION OF NEURON DAMAGE IN NEONATAL HYPOXIA USING MOLECULAR MARKERS. **Bu Coifiu Fanego R, Dorta Contreras AJ, Padilla Docal B, Noris García E, González Hernández M, Rodríguez Rey A (Cuba)**

PNI-08 PROLONGED SURVIVAL AND MIGRATION OF BONE MARROW-DERIVED STEM CELLS TRANSPLANTED INTO BRAIN LESIONS. **Alberti E, García R, Fraga JL, Serrano T, Hernández E, Klonisch T, Macías R, Martínez L, Castillo L, Los M, de la Cuétara K (Cuba)**

PNI-09 HAPTOBLOBIN/IGG INDEX VS SCORE´S BOYERI TO DIFFERENTIAL DIAGNOSIS OF THE MENINGITIS. **Gonzalez–Hernandez M, Noris-García E, Dorta Contreras A, Bucoifu-Fanego R, Fundora H, Padilla-Docal B (Cuba)**

PNI-10 TOXICOLOGICAL EVALUATION OF A NEW THERAPEUTIC OPTION IN NEUROLOGICAL PROCESSES, USING THE INTRACRANIAL INOCULATION. **Bacardi D, Cosme K, Bergado J, Pavón N, Suárez J, Vázquez A, Urquiza D, Romero J, Madrigal R, Aldana L, García Y, Bello I (Cuba)**

PNI-11 BONE MARROW STROMAL CELLS PRODUCING BDNF AND GDNF. **García-Miniet R, Pavón-Fuentes N, Vergara-Zubillaga P, Alberti-Amador E, Castillo-Díaz L, García-Varona A, Segovia-Vila J (Cuba)**

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PNI-12 PHYCOCYANIN EXERTS AN ANTI-INFLAMMATORY EFFECT ON THE CNS IN A MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS. **Martínez-Sánchez G, Pentón-Rol G, Valdivia-Acosta A, Oviedo-Gálvez M, Ramírez-Núñez O, Lagumersindez-Denis N, Cervantes-Llanos M, Falcón-Cama V, Acosta E, Rodríguez-Jiménez E, De Armas E, López-Saura P, Pentón-Arias E (Cuba)**

PNI-13 NEUROPROTECTIVE EFFECT OF DIETARY ZINC SUPLEMENTATION IN A TRANSGENIC MODEL OF SPINOCEREBELLAR ATAXIA SCA-2. **Batista Z, García JC, Cruz Y, Fernández M (Cuba)**

PNI-14 EFFECT OF THE AQUEOUS *Mangifera indica* L. EXTRACT ON MOTOR CONTROL OF SPINOCEREBELLAR ATAXIA TYPE 2 (SCA2) TRANSGENIC MICE. **Lemus-Molina Y, Martín Y, Amador A, Heredia L, Merino N, Delgado R (Cuba)**

PNI-15 ADMINISTRATION OF *Mangifera indica* L. STEM BARK EXTRACT AND MANGIFERIN IMPAIRS AVERSIVE MEMORY BUT IMPROVES OBJECT RECOGNITION INDEX IN RATS. **Maurmann N, Pardo Andreu GL, de Farias CB, Delgado R, Roesler R (Brazil)**

PNI-16 ANTICONVULSANT EFFECTS OF AQUEOUS AND METHANOLIC EXTRACTS FROM *Phragmanthera capitata* AND *Spathodea campanulata* IN RATS. **Atsamo AD, Nguelefack TB, Watcho P, Dongmo AB, Dimo T, Kamanyi A (Cameroon)**

PNI-17 ANTIBODIES AGAINST NFLP AND NEUROTROPIC VIRUSES IN MULTIPLE SCLEROSIS. **Hernández E, Robinson-Agramonte MA, Reiber H, Cabrera JA (Cuba)**

PNI-18 MODELING THE ORIGIN OF THE DIFFUSION WEIGHTED MRI (DWMRI) SIGNAL FROM GRAY MATTER IN MULTIPLE SCLEROSIS. **Martínez-Cancino R (Cuba)**

PNI-19 MODELING THE EFFECT OF DEMYELINATION ASSOCIATED WITH MULTIPLE SCLEROSIS ON EEG AND BOLD SIGNALS. **Sotero RC (Cuba)**

PNI-20 INTENSIVE PHARMACO-SURVEILLANCE OF IFN a2B IN THE TREATMENT OF MULTIPLE SCLEROSIS. **Pérez L, Ramos AM, Cabrera JA, Echazábal N, Bobillo H (Cuba)**

PNI-21 GUIDES FOR THE USE OF HUMAN IMMUNOGLOBULIN (INTACGLOBIN®) IN THE TREATMENT OF MULTIPLE SCLEROSIS. **Ramos AM, Pérez L, Cabrera JA, Echazábal N, Bobillo H, Fernández JD (Cuba)**

PNI-22 NEUROIMMUNOLOGY IN PATIENTS WITH MENINGOENCEPHALITIS CAUSED BY *Neisseria meningitidis* VACCINATED AND NOT VACCINATED WITH VA-MENGOC-BC VACCINE. **Noris García E, Dorta Contreras A, Bu Coifu Fanego R, González Hernández M, Padilla Docal B, Rodríguez A (Cuba)**

PNI-23 EVALUATION OF THE EFFICACY AND TOLERANCE OF THE NEUROLOGIC RESTORATION PROGRAM (NRP) IN PATIENTS WITH TRAUMATIC MEDULAR LESIONS. **Zamora F (Cuba)**

PNI-24 MODULATION OF THE EFFECT OF BAD NEWS IN THE ETHIOPATHOGENIC COURSE OF MULTIPLE SCLEROSIS. **Romero García KM, Cabrera Gómez JA, Real González Y, Bautista Pacheco K, Castro Armas L, Lima Machado C, Paixão Rocha M, Maris Rodríguez L (Cuba)**

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ABSTRACTS

PLENARY LECTURES (PL)
SATURDAY, APRIL 19

PL01- THE AUTOIMMUNE PATHOGENESIS OF MULTIPLE SCLEROSIS

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Multiple sclerosis is complex in more than one respect. Its clinical manifestation, a composite of motor, sensible and sensory defect and its unpredictable course are determined by a multitude of number, localization and activity of circumscript lesions distributed throughout the central nervous system. As hallmarks, MS lesions show inflammatory along with degenerative changes. According to a plausible concept, MS lesions are caused by an autoimmune response, where CNS specific T cells are activated within the peripheral immune system, invade the CNS and there start to attack local tissue components. The autoimmune attack, which may involve, besides T cells, B cells and macrophages, results in the destruction of periaxonal myelin sheaths, and the degeneration of local axons. This autoimmune concept of MS is based on observations collected from studies of human material as well as from experimental animal models. This presentation will review recent developments in the field.

PL02- DRUG-INDUCED IDIOSYNCHRATIC HEPATIC REACTION

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Drug-induced allergic hepatitis is a liver-specific inflammatory reaction as a consequence of a hypersensitivity reaction to a particular drug. Although less common than other forms of drug-induced hepatotoxicity, they have potentially more serious clinical implications, the outcome can be sometimes fatal. There is convincing experimental evidence to implicate the immune system in the pathogenesis of many drug idiosyncratic reactions. The classical view of this phenomenon identifies the formation and presentation of drug–protein adducts (and/or a direct interaction with the major histocompatibility complex/T-cell receptor complex) as an *initiating* factor to trigger a hypersensitivity reaction. This is followed by T-cell proliferation and cell-mediated liver injury. Despite the formation of drug-protein adducts is not uncommon, liver shows tolerance against them. Indeed, an allergic hepatitis occurs only when this tolerance is impaired. It is not clear what determines this loss of tolerance towards drug antigens. Epiphenomena such as cell injury caused by the drug itself, a concomitant inflammatory process, or a coincidental stimulus seem to be needed to break this tolerance. The allergic hepatitis induced by drugs is generally a Type IV hypersensitivity reaction involving CD4+, CD8+ cytotoxic lymphocytes as well NK cells. Antibodies directed to the drug are much less common. Drug allergic hepatitis is frequently associated with fever, rash and liver cell infiltration (DRESS syndrome). In few cases damage to liver cells may perpetuate in the form of an autoimmune hepatitis. The available diagnostic tools to confirm the involvement of a given drug in an immune-mediated hepatic injury are rather limited, and this is largely due to a still incomplete understanding of the pathogenesis of drug allergy in the liver.

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PL03- IMMUNOTHERAPY OF CANCER: MORE THAN JUST ANTIBODIES AND VACCINES; THE IMPORTANCE OF SYNERGIE AND SEQUENTIAL MANAGEMENT IN CANCER

Dalgleish A

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Cancer vaccines have been studied for several decades and have been associated with improvements in identifying tumour antigens and new technologies over the last few years. Very encouraging phase II data in melanoma, in particular, has not been confirmed by large randomised studies. Whereas most of the early studies have focussed on melanoma because of its perceived immunological sensitivity, it has become apparent that the other solid tumours may in fact be a better target. Indeed, the only positive clinical data in randomised studies to date have occurred with autologous tumour based vaccines in renal and colorectal cancer. A number of different technological approaches have shown very encouraging data with regards to prostate cancer and both lung and breast have very encouraging candidates.

Although adaptive immunity is clearly required to control antigen specific expression, it has become apparent that when specific tumour antigens are targeted they can downregulate rapidly, particularly with regards to melanoma.

More recently, attention has turned to the potential beneficial role of the innate immune system in immunity and a number of different approaches are being employed to exploit this.

One of the issues that has come to light is the potential importance of sequential management in the overall outcome. It has been known for many years that patients who have had some form of immunotherapy appear to respond better to radiotherapy and our own group has recently reported this to be the case for patients primed with Imiquimod and low dose IL-2. It is also apparent that the outcome for subsequent endocrine therapy may also be better in the case of prostate cancer and chemotherapy in a number of circumstances. Indeed, the failure of the prostate cancer vaccine of Dendreon Corporation to meet any of its primary criteria but to show an increased overall survival, compared to the controls, may well be due to the fact that the vaccine may well be sensitising the patients to respond to the Doxytaxotere, which they all go upon relapsing.

This raises the question of potential synergy with other treatments and, indeed, there is a large body of pre clinical data showing potential synergy with anti inflammatories, anti angiogenics, radiotherapy and some forms of chemotherapy. It is possible that the synergy with chemotherapy involves the ability to inhibit T-regulartory cells, as is the case for low dose Cyclophosphamide, or myeloid suppressor cells, as is the case for Gemcitabine.

Even the presence of a perfect immune response, there is still the problem of tumour escape and tumour induced suppression, which can take the form of either expressing complimentary K accelerating factor-like ligands, e.g. CD55 and CD59, or the secretion of immunosuppressive cytokines, such as TGFβ and IL-10. A number of strategies to overcome this have been developed.

In conclusion, the failure of cancer vaccines to become registered and part of the main stream of cancer treatment has, up to now, been due to the very high barriers placed on the successful outcome by often trialing the vaccine singularly and in unsuitable tumour stage populations. The ability to identify patients that will respond to this form of therapy and identify a strong sequential management therapy (SMT) programme to optimise the benefit of immune stimulation would be the route for future success.

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PLENARY LECTURES (PL) OR LECTURES (L)
SUNDAY, APRIL 20

PL04- COMBINING SUPPRESSIVE AND IMMUNO-STIMULATORY SIGNALS ON PROTEOLIPOSOMES: A NEW APPROACH FOR IMMUNE COMPROMISED SCENARIOS

Mesa C, de León J, Fernández A. Mazorra Z, Oliver L, Peña V, Sánchez B, Carr A, Fernández LE.

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Vaccine design for cancer immunotherapy has to deal with self and highly tolerated antigens as well as with immune-compromised patients. In this sense a rational and unconventional design of adjuvants and immune-potentiators needs to be performed. Here we described a new approach in which NAcGM3 ganglioside, with pronounced immune-suppressive effect, is incorporated into the outer membrane complex of *N. meningitidis* to form Very Small Size Proteoliposomes (VSSP). Here we demonstrate that VSSP, independently of the predominance of the ganglioside in their structure, provide the necessary "danger" signals to provoke the immune system activation. These studies reveal VSSP as a promoter of DC maturation and induction of inflammatory cytokines in both mouse and human DC, but also on VSSP treated melanoma patients. This effect is mediated by VSSP and TLR4 and TLR2 interaction. The subsequent activation of adaptive immune system showed a Th1 polarized system. The capacity of VSSP to promote cross-priming and antigen specific CD8 CTL responses was also demonstrated. These nanoparticles, demonstrated strong adjuvant effect for a wide range of low-immunogenic antigens with a subsequent induction of anti-tumor responses in different murine tumor models. Moreover, VSSP, in its interaction with the immune system, interfere with the negative effect of an immune-suppressive molecule, the NAcGM3 ganglioside, used by tumors as a mechanism to escape the immune-surveillance. This peculiarity is probably the reason by which VSSP could show also effectiveness on immune-compromised scenarios such as AIDS and cancer patients even under treatment with high doses of chemotherapy.

PL05- CD36 AS MEDIATOR OF THE ANTI-INFLAMMATORY AND ANTI-ATHEROSCLEROTIC PROPERTIES OF GROWTH HORMONE-RELEASING PEPTIDES (GHRPS)

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Introduction. A chronic treatment with EP80317, a selective CD36 ligand belonging to the growth hormone-releasing peptides (GHRPs) family, is associated with striking anti-atherosclerotic effect in apoE$^{-/-}$ mice. This study aimed to elucidate the mechanisms underlying the anti-atherosclerotic action of GHRPs. **Methods**. ApoE$^{-/-}$ mice were treated with EP80317 (300 µg/kg daily, sc) for 6 or 12 weeks. The effect of GHRPs on mononuclear cell recruitment to the aortic wall was assessed by monitoring ^{111}In-labeled peritoneal macrophages (mac) 24 h following the i.v. injection of labeled cells into apoE$^{-/-}$ mice fed a high cholesterol diet. Aortas were collected for determining the aortic mRNA levels of inflammatory biomarkers. The effect of GHRP on oxLDL-elicited phosphorylation of p125FAK and pyk2 in mac was also assessed. **Results**. EP80317 reduced by 65% mac trafficking to the aortic intima, in accordance with a 42% reduction in oil red-O-stained aortic lesion areas,

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suggesting that EP80317 interferes with oxLDL-elicited monocyte recruitment. In support, incubation of monocytic cells with EP80317 reduced the phosphorylation of p125FAK and pyk2, key kinases involved in mononuclear cell trafficking. In contrast, labeled mac lacking CD36 expression were not prevented to accumulate to lesions. GHRP reduced the expression of vascular VCAM-1, MCP-1, gp91phox and iNOS by ~ 50-60% ($p < 0.05$). **Conclusion**. GHRP reduces 1) mononuclear cell accumulation within the aortic vascular wall in a CD36-dependent manner 2) the expression of inflammatory vascular aortic wall biomarkers including VCAM-1, MCP-1, NADPH oxidase and iNOS. These findings might explain, at least in part, the anti-atherosclerotic properties of CD36 ligand. Supported by the Canadian Institute of Health Research

PL06- T-CELL IMMUNOREGULATION IN MULTIPLE SCLEROSIS. EFFECT OF C-PHYCOCYANIN AND ITS COMBINATION WITH ALPHA INTERFERON

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Introduction: Multiple Sclerosis (MS) is the most frequent autoimmune central nervous system demyelinating disease with severe and often devastating relapse and remitting attacks. MS relapses and remissions indicate that even during relapses there are control mechanisms triggered. Experimental autoimmune encephalomyelitis (EAE) studies in rodents suggest that immunological acute attacks are self-limited by rTc. We demonstrate a new property of C-Phycocyanin (C-Pc), a major biliprotein of the blue-green algae *Spirulina platensis*. **Materials & Methods:** Peripheral blood mononuclear cells (PBMC) were separated from MS patients' peripheral blood. The cells were treated with: IFN-alpha, C-Pc, IFN-alpha/C-Pc and untreated cells. The genes of cTr markers were amplified and the CD4+CD25highFoxp3+ subset of different experimental groups was measured by flow cytometry. We also evaluate the effect of systemic C-Pc administration on Lewis rats where EAE was induced. Severity of clinical signs and redox biochemical biomarkers were measured. **Results:** We found that in PBMC from MS patients and HC "in vitro" treated with IFN-alpha, C-Pc and its combination provokes a CD4+CD25high+Foxp3+ rTc response. We also demonstrated that C-Pc is also able to trigger more basic underlying mechanisms that lead to prevent EAE induction if previously administered to rats or downgrade EAE if given after it has been established. Transmission electron microscopy studies also showed the myelin and axonal damage improvement induced by C-Pc treatment. **Conclusions:** Our results show an induction of rTc, suggesting that this subset may mediate the C-Pc effect. These results agree with reports indicative of that rTc limits acute MS attacks. Other autoimmune and neurological diseases could also benefit from these findings.

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1st International Workshop of ImmunoPharmacology

L01- ACTIVATION OF NATURAL NEUGC-CONTAINING GANGLIOSIDES RELATED IDIOTYPIC NETWORKS IN NSCLC PATIENTS IMMUNIZED WITH AN ANTI-IDIOTYPE ANTIBODY

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1E10 mAb is an anti-idiotype murine monoclonal antibody (Ab2 mAb) specific for an Ab1 mAb which reacts with NeuGc-containing gangliosides, sulfatides and antigens expressed in some human tumors. In pre-clinical studies, this Ab2 antibody was able to mimic NeuGc-containing gangliosides only in animals lacking expression of these antigens in normal tissues and showed potent antimetastatic effects in murine models of melanoma and lung carcinoma. In this study, we report on the immune responses elicited in 20 NSCLC patients treated with aluminum hydroxide-precipitated 1E10 mAb. In the hyperimmune sera of the patients, a strong specific antibody response of both IgM and IgG isotypes against NeuGcGM3 ganglioside was observed. Patient immune sera were able to induce complement-independent cell death of NeuGcGM3-expressing X63 murine myeloma cells. Significant immunoreactivity to NeuGcGM3 was still detected after the complete abrogation of the reactivity against 1E10 mAb by the adsorption of patient sera with this antibody. These Id-Ag+ antibodies suggest the induction of Ab5 antibodies by the activation of an autologous idiotypic cascade into the patients. Both Id+Ag+(Ab3) and Id-Ag+(Ab5) fractions, were separated by affinity chromatography and characterized. While IgG isotype antibodies were found in both Ab3 and Ab5 fractions, IgM isotype antibodies were found only in the Id-Ag+ Ab5 fraction. These Ab5 antibodies were able to specifically recognize and induce cell death in NeuGcGM3-expressing X63 myeloma target cells. In our study, those patients that developed IgG and/ or IgM antibodies against NeuGcGM3 showed longer median survivals times (14,265 vs 6,35 months), ($p<0.05$, Mann-Whitney test, one tail).

L02- 1E10 ANTI-IDIOTYPE VACCINE IN NON-SMALL CELL LUNG CANCER. EXPERIENCE IN STAGE IIIB/IV PATIENTS

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Conventional treatment of non-small cell lung cancer (NSCLC) has apparently reached a plateau of effectiveness in improving the survival of the patients. For that reason the search for new therapeutic strategies in this type of tumor is justified. 1E10 is an anti-idiotype murine monoclonal antibody (Ab2 MAb) specific to P3 Ab1 MAb, which reacts with NeuGc-containing gangliosides, expressed in the lung tumors. We report the treatment with aluminum hydroxide precipitated 1E10 MAb of 34 stage IIIb and 37 stage IV NSCLC patients. These patients were treated with the anti-idiotype vaccine, after received standard chemotherapy and radiotherapy, in a compassionate-use basis study. Patients received five bi-weekly injections of 1 mg of 1E10/Aluminum, other 10 doses at 28-day intervals and later the patients who maintained a good performance status continued to be immunized at this same time interval. No evidence of unexpected or serious adverse

effects was reported. The median survival time of the 56 patients who entered the study with partial response or disease stabilization and with a PS 1 after the first line of chemo/radiotherapy, was 11.50 months from starting vaccination. In contrast, the median survival time calculated for patients who started vaccination with progressive disease and/or a PS2 was 6.50 months. A preliminary evaluation of the effect of vaccination on survival was performed by comparing survival of vaccinated patients with a nonrandomized control group. The median survival time of these controls was significantly lower. A randomized phase II study in patients with stage IIIB/IV NSCLC having achieved CR/PR/SD after standard front line therapy is ongoing.

L03- TREATMENT OF HIGH-GRADE GLIOMA PATIENTS WITH THE HUMANIZED ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MONOCLONAL ANTIBODY NIMUTOZUMAB

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The poor prognosis of patients with high-grade glioma has led to the search for new therapeutic strategies. More than half of these tumors overexpress Epidermal Growth factor Receptor (EGFR). Nimotuzumab is a humanized monoclonal antibody that recognizes the EGFR external domain with high affinity, inhibiting tyrosine kinase activation. In order to evaluate safety, immunogenicity and preliminary efficacy of Nimotuzumab in newly diagnosed high-grade glioma patients, we conducted a Phase I/II trial. Patients received six weekly infusions of Nimotuzumab at the dose of 200 mg in combination with external beam radiotherapy. Twenty-nine patients were entered into the study. Tumor types were: glioblastoma (GB) (16 patients), anaplastic astrocytoma (AA) (12 patients) and anaplastic oligodendroglioma (AO) (1 patient). All patients underwent debulking surgery or biopsy before entering the trial. The antibody was very well tolerated. No evidences of grade 3/4 adverse events were detected. None of the patients developed acneiform rash or allergic reactions. Objective tumor response-rate was 37.9% (17.2% complete response, 20.7% partial response) while stable disease occurred in 41.4% of the patients. With a median follow up time of 29 months, the median survival was 22.17 months for all subjects. Median survival time (MST) was 16.30 months for GB, whereas MST is not reached for AA patients. Resent data from an ongoing controlled phase III trial tend to confirm the results obtained from this previous trial.

L04- RELEVANCE OF MONOSYALYL LACTOSYL CERAMIDES IN CANCER: THEIR INFLUENCE ON TUMOR IMMUNOSURVEILLANCE

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Accumulated evidences suggest a role of gangliosides in tumour progression. These molecules can be shed, both at the tumour site and at secondary lymphoid organs, contributing to the cancer immune surveillance avoidance. Significantly, certain human tumours express the N-glycolylated variant of GM3, a rather immunogenic ganglioside which is practically undetectable in normal human tissues. We explored whether the expression of this molecule means any advantage for tumour progression, assessing its influence on T and dendritic cells. This work demonstrates the capacity of NGcGM3 ganglioside to down-modulate CD4 expression in murine and human T lymphocytes, especially in non-activated T cells. After recovering the CD4 expression, T cells kept a similar sensitivity to ganglioside-induced CD4 down-modulation. In addition, a clear association between NGcGM3 insertion in lymphocyte plasma membranes and the CD4 down-modulation effect was detected. The impact of this molecule on both regulator and effector $CD4^+$ T cells was also compared,

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demonstrating that NGcGM3 mainly affects $CD4^+CD25^-$ T cell proliferation, promoting secretion of anti-inflammatory cytokines. Noteworthy, the inhibitory role of regulatory T cells was not modified by the ganglioside. Furthermore, NGcGM3 inhibits dendropoiesis and hampers LPS-induced DC maturation. The role of the NGcGM3 ganglioside in tumour progression was also outlined. Reduction in the ganglioside content significantly affected the tumour growth of X63 myeloma cells, inoculated in Balb/c mice. This effect was abrogated in CD4+ T cells depleted mice. Overall, these results highlight the relevance of NGcGM3 for tumour progression and reinforce the interest in this molecule as a target for cancer immunotherapy.

L05- A NOVEL MECHANISM IN α ENaC REGULATION IDENTIFIED BY MOLECULAR CLONING, EXPRESSION AND CO-EXPRESSION OF α ENaC ALTERNATIVELY SPLICED FORM "b" AND α ENaC WILDTYPE

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INTRODUCTION: In Dahl rats' kidney cortex, the salt-sensitive alternatively spliced form of the epithelial sodium channel α subunit (α ENaC b) is the most abundant mRNA transcript (32+/-3 fold > α ENaC wt) as was investigated by quantitative RT-PCR analysis. α ENaC b mRNA levels were significantly higher ($P<0.05$) in Dahl R *versus* S rats (Shehata et al., 2007). **OBJECTIVES:** In the present study, we described the molecular cloning and searched for a possible role of α ENaC b by testing its potential expression in COS7 cells as well as its impact on α ENaC wt expression levels when co-expressed in COS7 cells in a dose-dependant manner. **METHODS:** Using RT-PCR strategy, the full-length wildtype α ENaC transcript and the alternatively spliced form α ENaC b were amplified, cloned, expressed and co-expressed into COS7 cells in a dose dependant manner. A combination of denaturing and native western blotting techniques were employed to examine the expression of α ENaC b in vitro, and to determine if an interaction between α ENaC b and α ENaC wt occurs in vitro, and finally to demonstrate if degradation of α ENaC wt protein does occur. **RESULTS:** α ENaC b is translated in COS7 cells. Co-expression of alternatively spliced α ENaC b form together with α ENaC wt reduced α ENaC wt levels in a dose dependant manner. α ENaC wt and b form appear to form a complex that enhances the degradation of α ENaC wt. **CONCLUSIONS:** Western blots suggest a novel mechanism in α ENaC regulation whereby α ENaC b exerts a dominant negative effect on α ENaC wt expression. This is potentially by sequestering α ENaC wt, enhancing its proteolytic degradation, and possibly explaining the mechanism of salt-resistance in Dahl R rats.

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L06- EVALUATION OF THE EFFECTS OF *Zataria multiflora*, *Geranium pelargonium*, *Myrth* AND *Lemon* ESSENCES ON IMMUNE SYSTEM FUNCTION IN EXPERIMENTAL ANIMALS

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The effects of some Iranian herbal essences have been evaluated on the function of immune system using experimental animals. Rabbits received *Zataria multiflora*, *Geranium pelargonium*, *Myrth*, *Lemon* essences and normal saline (control group), 6 times with 6 days of interval. Five days after the last injection of the essences, *Candida albicans* antigens were injected into all the animals. Phagocytosis and killing assays and lymphocyte transformation test (LTT) were carried out on blood samples. The cellular immunity was significantly stimulated against *C. albicans* antigens and Con-canavalin A (Con-A) mitogen in animals that injected subcutaneously with *Z. multiflora* and *G. pelargonium* in comparison with the control group, whereas *Myrth* essence had no considerable effect and *Lemon* essence suppressed the cellular responses. *Zataria multiflora*, *Myrth* and *Lemon* essences stimulated innate immunity when injected subcutaneously, whereas *G. pelargonium* essence had no significant effect. Humoral responses to *Candida* antigens were significantly decreased in animals injected with *Lemon* essence as compared to other essences ($p< 0.05$).

L07- IMMUNOSUPPRESSIVE AND PHYTOCHEMICAL PROPERTIES OF *Tinospora bakis* Miers PLANT ETHANOLIC EXTRACT

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The crude 80% ethanolic/water extract of *T. bakis* Miers braches was investigated for its immunomodulating activity using luminol/lucigenin based-chemiluminescence assay on polymorph nuclear cells (PMNs), mononuclear cells (MNCs) and macrophage cells (MQ) oxidative burst activity. The plant crude extract showed inhibitory activity for PMNS and MNCs ranging between 53 – 94% when serum opsonized zymosan-A (SOZ) used as cell stimulator for the concentrations 6.25 – 100 µg/mL. Less inhibitory activity than (< 42%) was observed for MQ with above tested concentrations. This activity was completely disappeared when SOZ was substituted with PMA, a protein kinase C (PKC) activator. In a separate experiment the plant extract was found more potent inhibitor for T lymphocyte proliferation assay (IC_{50} = 10 µg/mL). The phytochemical investigation led to isolation of two major terpens in bulk amount columbin at yield of 2.6 X 10^{-1}% and β-Sitosterol at yield: 1.1 X 10^{-4}% but they didn't show any potent immunosuppressive activity.

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5th International Workshop of Inflammation and Pain

L08- BIPHASIC ROLE FOR LYMPHOCYTES IN ACUTE INFLAMMATION AND ITS RESOLUTION

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Acute inflammation is traditionally described as the influx of PMNs followed by monocyte-derived macrophages leading to resolution. This is a classic view and despite sub-populations of lymphocytes having innate immune-regulatory properties, seldom is the role of lymphocytes in acute inflammation and its resolution discussed. To redress this issue we show that during onset of acute peritonitis resident peritoneal T and B lymphocytes control PMN trafficking by regulating cytokine release. Once inflammation ensues, lymphocytes disappear in response to DP1 receptor activation by prostaglandin D_2 (PGD_2). However, as inflammation resolves a different proportion of lymphocytes to that in the naïve state repopulate the cavity comprising B1 cells along with NK cells, gamma/delta T cells, $CD4^+/CD25^+$ cells and B2 cells. Importantly, repopulating lymphocytes do not bring about resolution but protect against super-infection such that their absence during non-resolving inflammation predisposes to exaggerated inflammatory responses upon secondary challenge. Therefore, we describe a transition in lymphocyte populations from onset of acute inflammation to resolution, which is under the control of PGD_2 and has a crucial role in modulating early responses to injury and conferring protection against secondary infection. These findings highlight the importance of lymphocytes along with granulocytes in mounting appropriately-tempered innate immune-mediated responses.

L09- A THERAPEUTIC ROLE FOR CANNABINOIDS IN THE MANAGEMENT OF ARTHRITIS PAIN AND INFLAMMATION

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Introduction: Arthritis pain and inflammation is one of the leading causes of disability in the world for which there is still no ideal treatment (1). Cannabinoids are a family of alkaloids derived from the hemp plant *Cannabis sativa* which are known to modulate multiple physiological functions including pain (2). The present study examined whether the synthetic cannabinoid ACEA (arachidonyl-2'-chloroethylamide) could modulate joint nociception and blood flow in the rat knee. **Materials & Methods:** Joint blood flow was measured in response to topical application of ACEA (10^{-14} – 10^{-9} mol) onto male Wistar rat knees. In a separate cohort, osteoarthritis (OA) was induced by intra-articular injection of sodium monoiodoacetate (14 days recovery) and joint nociception measured by electrophysiologically recording from knee joint primary afferents in response to mechanical rotation of the knee. Non-noxious and noxious movements were made before and following close intra-arterial injection of different doses of ACEA (10^{-10} to 10^{-7} mol). The effect of the cannabinoid CB_1 antagonist AM251 and transient receptor potential vanilloid-1 (TRPV1) antagonists on ACEA-mediated vasomotor and nociceptive responses was also assessed. **Results:** ACEA caused a dose-dependent increase in synovial blood flow. In OA knee joints, application of ACEA caused a significant reduction in afferent firing rate during normal and noxious rotation of the knee joint. Blockade of peripheral CB_1 receptors and TRPV1 channels significantly attenuated the antinociceptive effects of ACEA. The hyperaemic response to ACEA was inhibited by TRPV1 antagonism only. **Conclusions:** Local administration of the cannabinoid ACEA caused vasodilatation in the rat knee joint. In addition, treatment of OA knees with ACEA reduced nociceptive activity suggesting that cannabinoids have the potential to relieve OA pain. A novel mechanism was uncovered wherein the

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physiological effects of ACEA appeared to be mediated via the TRPV1 channel. **References: 1)** McDougall, JJ. Arthritis and Pain. Neurogenic origin of joint pain. Arthritis Research and Therapy 2006;8(6):220-229. **2)** Schuelert, N and McDougall, JJ. Cannabinoid-mediated antinociception is enhanced in rat osteoarthritic knees. *Arthritis and Rheumatism* 2008;58(1):145-53.

L10- HYDROGEN SULFIDE-RELEASING DRUGS: A NEW CLASS OF ANTI-INFLAMMATORIES

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs, but their use is associated with clinically significant gastrointestinal ulcers and bleeding in 2-4% of patients. Hydrogen sulfide (H_2S) has recently been demonstrated to be an endogenous mediator of gastric mucosal defence, as well as exerting significant anti-inflammatory effects. Moreover, we recently reported that H2S can accelerate the healing of pre-existing ulcers.
We have synthesized a series of H_2S-releasing anti-inflammatory drugs. These drugs include derivatives of mesalamine (for treatment of inflammatory bowel disease) and naproxen (for treatment of arthritis). In both cases, the H2S-releasing derivatives exhibit significantly enhanced anti-inflammatory actions in animal models. In the case of the naproxen derivative (ATB-346), no gastrointestinal injury was observed following its administration to rats twice-daily for five days at a dose of 50 umol/kg, whereas an equimolar dose of naproxen caused widespread ulceration. However, ATB-346 inhibited gastric prostaglandin synthesis as effectively as naproxen. The lack of ulcerogenic effects of ATB-346 were not lost when the drug was tested in rats pre-treated with a nitric oxide synthase inhibitor (L-NAME), or with an inhibitor of endogenous H_2S synthesis (BCA). Moreover, the gastric tolerability was maintained when given to rats in which capsaicin-sensitive sensory afferents had been ablated.
Our studies suggest that hydrogen sulfide-releasing derivatives of mesalamine and naproxen are superior anti-inflammatory drugs as compared to the parent drugs, both in terms of efficacy and tolerability.

L11- A *LIMULUS*- $LALF_{32-51}$ DERIVED PEPTIDE MODULATES THE INFLAMMATORY RESPONSE IN MACROPHAGES EXPOSED TO LIPOPOLISACCHARIDE

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Introduction: The immune system is characterized by the ability to respond to infectious agents without producing a destructive response against self-body. We showed that a *Limulus* anti-LPS Factor derived peptide has novel biological properties comprising anti-inflammatory and immunomodulatory capacities. The effectiveness shown by this peptide, in different models of bacterial infection, can be due to a selective up-regulation of elements of innate immunity, which avoid or limit damages caused by inflammation. **Material & Methods:** The monocytic leukemia cell line THP-1 (ATCC TIB 202). The peptide was synthesized manually using Fmoc/tBu solid phase. EMSA was performed using the Gel Shift Assay System. Cytokines and TLRs RNA levels were analysed by reverse-transcribed, and cDNA was amplified using GeneAmp RNA PCR kit. The production of TNF-α was measured using an ELISA assay. **Results:** Our findings demonstrate that the $LALF_{32-51}$ peptide promotes THP-1 cell differentiation to macrophage with a particularly phenotype resulting in the

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abrogation of LPS-induced TNF-α gene expression and the up-regulation of the gene for the TGF-β cytokine. On the other hand, we report the up-regulation of the genes of the Toll-like receptors in THP-1 derived-macrophages by the $LALF_{32-51}$ peptide. Furthermore, band shift analysis revealed the down-regulation of the transcriptional factor NF-κB in peptide-treated cells challenged with LPS. **Conclusion:** Our results suggest that the $LALF_{32-51}$ peptide induces an anti-inflammatory phenotype in THP-1 derived- macrophages without impairing their susceptibility to pathogens by up-regulating the expression of several TLRs. In consequence, this molecule combines the unique properties of an anti-infectious and an anti-inflammatory factor.

L12- A SIGNIFICANT DECREASE OF SERUM IL6 AND MMP3 AFTER ETANERCEPT TREATMENT IN PATIENTS WITH ANKYLOSING SPONDYLITIS

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Introduction: We measured the serologic level of many cytokines before and after etanercept treatment for severe ankylosing spondylitis in order to understand which cytokines may serve as a marker to evaluate the treatment outcome. **Methods:** Within one year, 16 AS patients received etanercept treatment in different medical centers. Before and after etanercept treatment (1M, 2M, 3M), 5cc venous bloods were obtained. The cytokines included TNF-α, IL1, IL6, IL8, IL10, TGF-β, TRAIL and MMP. The serum levels were measured by ELISA test. In addition, we used flow cytometry method to measure toll-like receptor 2 and 4. **Results:** We could not detect IL1 and IL8 in all cases before and after etanercept treatment. There was no significant change for IL10, TGF-β, TRAIL. TNF-α could not be detectable in the baseline but after etanercept treatments over 2 weeks, it significantly increased in all patients but there was no significant change between each month. IL6 in the serum is not very high in baseline but it could be significantly reduced after etanercept treatment (baseline 14.2±14.5 vs 3M 4.0±2.8, p=0.001). Like IL6, MMP3 also decreased significantly after etanercept treatment (baseline 42.2±29.7 vs 3M 28.6±26.5, p=0.005). Peripheral blood CD14 only declined statistically at 4 months after etanercept treatment. Although TOLL-4 was decreased after 3 months treatment, there was no significant difference. **Conclusion:** IL6 and MMP3 may be used as a marker to evaluate the treatment outcome of etanercept in patients with severe AS.

L13- ANTINOCICEPTIVE EFFECTS OF GUANOSINE IN MICE: EVIDENCES FOR THE MECHANISM OF ACTION

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It is well known that adenine-based purines exert multiple effects on pain transmission. However, less attention has been delivered to guanine-based purines (guanosine). This study investigated the antinociceptive effects of i.c.v., i.t., or systemic (acute or chronic) administration of guanosine on several animal pain models. Additionally, the mechanism of action of guanosine was investigated through several measures including pharmacological reversion by receptor antagonists and glutamate uptake on brain and spinal cord slices. Briefly, this study showed that guanosine presents prominent antinociceptive effects and provides new evidence on the mechanism of action of guanosine. These effects seem to be not mediated by adenosine or opioid receptors and might be related, at least partially, to modulation of the glutamatergic system by guanosine.

IMMUNOPHARMACOLOGY 2008
1st International Workshop of ImmunoPharmacology
5th International Workshop of Inflammation & Pain
1st International Workshop of NeuroImmunology
1st International Symposium about Pharmacology of Cytochrome P450
Varadero, Cuba. April 19-22, 2008
http://www.scf.sld.cu/impharmacology08/impharmacology08.htm

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L14- ANALGESIC PROFILE OF VIMANG TABLETS IN PATIENTS WITH CHRONIC PAIN

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It has been accepted that neuroinflammation, oxidative stress and glial activation play a roll in the central sensitization underlying in the neuropathic and inflammatory chronic pain. Vimang is the brand name of an aqueous extract of *Mangifera indica* L. traditionally used in Cuba for its anti-inflammatory, analgesic, antioxidant and immunomodulatoy properties. In the present study, we determined the effects of Vimang's tablets and other formulations in persons with chronic pain. The Complex Regional Pain Syndrome (CRPS) (n=15) and Pain Associate with Zoster patients (n=12) received a daily dose of 1800 mg of extract (three coated Vimang tablets, 300 mg each, before meals, and cream 1.2% as topical agent for 120 and 90 days, respectively). Compress containing the dissolution 2 % was utilized on skin lesions in 6 Herpes Zoster (HZ) patients for 30 days. In knee osteoarthritis (OA) patients (n=10), 3 groups were design (n=3 for groups); group 1 received a daily dose of 1800 mg (tablets), group 2 received 900 mg (tablets) and group 3 received a combination of tablets 900 mg and cream for 90 days. The change in average daily pain daily score through a Likert scale, the area and rate of dynamic allodynia, rate of thermal allodynia, and burning spontaneous pain frequency were evaluated. The deep somatic allodynia and joint's function in CRPS patients were evaluated, too. The WOMAC Index (Western Ontario and McMaster Universities) was utilized in knee OA and evaluated the presence of synovitis and effusion for ultrasonography. From week 2 pain scores and sensory abnormalities showed a significant greater improvement. The joint's function increased in CRPS patients. In acute Herpes Zoster patients, the analgesic effect was observed from week 1 and none developed post-herpetic neuralgia (PHN). In patients with persistent pain less than 3 months post HZ and PHN, pain scores and sensory abnormalities showed a significant improvement from week 4. The analgesic effect and improved joint's function were observed from week 2 associated with the decreased of the synovial effusion in kee´s patients with OA. Both effects were observed, the increase and decrease of synovial thickness (mm) independently of the analgesia. The inhibition of the proliferation was significant in the group 2. Our results suggest that Vimang's formulations might be appropriate to prevent and treat chronic pain.

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1st International Workshop of NeuroImmunology

L15- A PUTATIVE ROLE FOR BRAIN MARKERS

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A number of molecules and proteins have been suggested as markers of neuronal damage and glial injury/activation. Identification of biochemical markers of brain injury would represent a major step forward in the noninvasive assessment or monitoring of the efficacy of neuroprotective therapies. Moreover, biochemical brain markers have been used in both experimental and clinical studies. S100B protein, neuron-specific enolase, lactate and brain derived neurotrophic factor are considered sensitive brain markers since their levels are increased in the cerebrospinal fluid and/or blood in acute and chronic central nervous system (CNS) injuries. Thus, the cellular specificity of these proteins can be used to understand the involvement of different brain cell types in pathological conditions.

L16- INFLUENCE OF MELATONIN ON ADULT NEUROGENESIS FOLLOWING GLOBAL CEREBRAL ISCHEMIA IN SPRAGUE-DAWLEY RATS

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The experiment was designed to study the influence of melatonin on adult neurogenesis following global cerebral ischemia in Sprague-Dawley rats. Rats weighing between 300-400 gm were used for the study. All the rats were given 5 mg/kg of aqueous melatonin intra-peritoneally 30 minutes before the common carotid artery occlusion (CCAo) to established global cerebral ischemia. Six of the rats serve as sham control. The rats were euthanized 24 hours, 48 hours and 72 hours post ischemia, perfusion-fixed with cold 0.9 % saline followed by 4 % parafomaldyhyde and the brains were removed for immunocytochemistry staining for rats anti BrdU and anti proliferating cells protein K_i-67 markers for proliferating cells was done. Results of post ischemic recovery showed that the rats were very sluggish but no obvious limbs paralysis was observed. The brain surface showed distended and dilated blood vessels with hemorrhagic spots. Immunopositive BrdU and K_i-67 neurons were reviewed in the subventricular zone and dentate gyrus of the hippocampus. The role of melatonin in adult neurogenesis was discussed.

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L17- ASTROGLIAL AND COGNITIVE EFFECTS OF CHRONIC CEREBRAL HYPOPERFUSION IN THE RAT

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The permanent occlusion of common carotid arteries (2VO), resulting in a significant reduction in cerebral blood flow (hypoperfusion) in rats, is accepted as a well established experimental model to investigate neuronal damage and cognitive impairment that occurs in human ageing and Alzheimer's disease. In the present study, we evaluated two astroglial proteins: glial fibrillary acidic protein (GFAP) and S100B, a neurotrophic cytokine altered in many brain disorders. These proteins were measured in cerebral cortex and hippocampus tissue, as well S100B in cerebrospinal fluid. We also investigated glutamate uptake and glutamine synthetase activity in hippocampus, as well cognition evaluated by reference and working spatial memory protocols. Adult male Wistar rats were submitted to 10 weeks of chronic cerebral hypoperfusion after the 2VO method. A significant increase of S100B and GFAP in hippocampus tissue was observed, as well a significant decrease in glutamate uptake. Interestingly, we observed a decrease in S100B in cerebrospinal fluid. As for cognitive function, there was an impairment of both reference and working spatial memory in the water maze. Our data so support the hypothesis that astrocytes play a crucial role in the mechanisms of neurodegenerative disorders, like Alzheimer's disease, and that hippocampal pathology arising after chronic hypoperfusion gives rise to memory deficits.

L18- EVALUATION OF BLOOD-CEREBROSPINAL FLUID BARRIER IN GUILLAIN-BARRÉ SYNDROME

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Introduction. Elevated total protein concentration with normal CSF cell count is a hallmark for the diagnosis of Guillain-Barré syndrome (GBS), although protein levels may be normal during the first week. This work presents the evaluation of blood-CSF barrier (B-CSFB) dysfunction with the course, severity and clinical features of GBS. **Methods.** CSF was collected from 68 patients of both genders (15 children and 53 adults) who were admitted at the Institute of Neurology and Neurosurgery with the diagnosis of GBS. A second CSF sample was obtained approximately 15 days after treatment. Total protein concentration was determined in the CSF and 7.5% polycrylamide gel electrophoresis was employed for serum and CSF protein fractioning. **Results.** Elevated protein concentration and dysfunction of the B-CSFB was observed in more than 70% of the patients at admission, but this percentage was lower when the time from onset of disease was less than 10 days. There was a direct correlation between days from onset and CSF protein concentration and those patients with B-CSF B dysfunction had more days from onset and the clinical severity score was higher. A higher protein concentration was observed in the second CSF sample, with more patients displaying B-CSFB dysfunction. **Conclusions.** When evaluating CSF studies for diagnosis in GBS, time of evolution, clinical severity and phase of the disease must be taken into account.

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L19- IMMUNE RESPONSE IN CHILDREN WITH *Neisseria meningitidis* B MENINGOENCEPHALITIS

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Introduction. One of the elements could help for the immunization scheme is to study the dynamics of the immune response according to antibodies levels against the specific biologic agent in convalescents. **Material and methods**. 85 children suffering from *Neisseria meningitidis* serogroup B belongs to the 1984's outbreak were studied. It was the last epidemic before the successfully vaccination campaign with Cuban vaccines. Total serum antibodies against Cuban vaccine immunogen from children from 0, 1,2,3,6 months and one year posterior rid of hospital were quantified by ELISA. **Results**. At day 30, a light increment of total antibodies against immunogen was observed. There was a decrement of antibodies during the second month. Later on during the period from the third month up to the 6th month a continuous increment were appreciated with a posterior decrement of the antibodies level one year after the disease. **Conclusions**. There are two moments in which the antibodies levels diminished. This element could be of interest to analyse the immunization chart againt meningococus B.

L20- IMMUNO-QUANTIFICATION OF CYTIDINES AIMED AT THE DIAGNOSTIC OF NEURODEGENERATIVE DISEASES

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The trinucleotide repeat quantification procedures at low cost, represents a challenge for the molecular diagnosis aimed at massive screening of affected populations, especially neurodegenerative diseases. A new methodology for the detection and size estimation of CAG repeat expansions has been developed. The assay was based on PCR amplification from DNA model sequences, containing 20, 40 and 60 CAG repeats, for spinocerebellar ataxia types 2 genes. The PCR products were labeled with 6-(*p*-bromobenzamido)caproyl radical (BLC5), and Biotin, as reference, by means of transamination and acylation reactions. The label, which is specifically recognized by a monoclonal antibody, was separated from the nucleotide pyrimidine base by a spacer arm of 10 atoms. We obtained better results using the spacer arm larger. The amount of BLC5 specifically linked to cytidine nucleotides, in the labelled DNA molecules, was determined by Dot-Blotting assays and a calibration curve was carried out, as pattern, as well as, ELISA technique. The assay was simple, inexpensive, and easy to perform and differentiated distinct degrees of CAG expansions, due to the combined use of a specific labelling procedure for cytidine nucleotide and techniques considered as automated methods in quantitative immunotechnology.

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L21- CENTRAL NERVOUS SYSTEM DERIVED LIGHT A DECISIVE FACTOR FOR RECOVERY FROM EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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LIGHT is the latest identified ligand of the TNF family initially classified as a T cell positive costimulator. LIGHT promotes tissue damage; however, its costimulatory effect was recently shown to be redundant for the inflammatory response. Mice genetically deficient in LIGHT (*LIGHT*$^{-/-}$) were used to evaluate the contribution of this ligand in regulating CNS inflammation by using the rodent model experimental autoimmune encephalomyelitis. Following immunization with myelin oligodendrocyte glycoprotein (MOG), *LIGHT*$^{-/-}$ mice developed severe disease that resulted in an atypically high mortality rate. $CD4^+$ T cells from *LIGHT*$^{-/-}$ mice showed enhanced proliferation but reduced IFN-γ and IL-17 production compared to $CD4^+$ T cells from *LIGHT*$^{+/+}$ mice. Serum levels of reactive nitrogen intermediates and CNS transcripts of several proinflammatory chemokines were also substantially decreased in the absence of LIGHT. Adoptive transfer experiments and radiation chimeras indicated that expression of LIGHT on donor cells is not required for disease induction however its expression on nonhematopoietic derived host cells is a decisive factor to limit disease progression and tissue damage. Histologic examinations revealed intensive microglia/macrophages activation in the CNS and higher number of apoptotic cells within the CNS parenchyma of *LIGHT*$^{-/-}$ mice. Together, these data imply that LIGHT expression in the CNS plays a crucial role in controlling the immune response during autoimmune CNS inflammation.

L22- ASTROGLIAL S100B SECRETION IS STIMULATED BY IL-1β: ANOTHER PIECE IN THE "CYTOKINE CYCLE" PUZZLE OF ALZHEIMER'S DISEASE

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Background: S100B is an astrocyte-derived cytokine, which has been implicated in the IL-1β-triggered cytokine cycle in Alzheimer's disease. However, the secretion of S100B following stimulation by IL-1β has not been directly demonstrated. We investigated S100B secretion in glial preparations and hippocampal slices exposed to IL-1β. **Methods:** Cortical primary astrocyte cultures, C6 glioma cells and acute hippocampal slices from the brain of Wistar rats exposed to IL-1β (from 1pg to 10ng/mL) were utilized. Extracellular S100B was measured by ELISA. Nuclear migration of NF-κB was investigated by immunocytochemistry and immunoblotting. S100B secretion was also evaluated in the presence of specific inhibitors for NF-κB and MAPK. Statistical analyses were carried out by Student t test or one-way ANOVA, assuming $p < 0.05$. **Results:** IL-1β-stimulated S100B secretion was observed in all preparations. Nuclear migration NF-κB was also observed under these experimental conditions. PDTC (an inhibitor of NF-κB) and PD 98059 (an inhibitor of ERK) inhibited S100B secretion. S100B, at micromolar levels, like IL-1β, was able to induce NO production. **Conclusions:** Our data demonstrate IL-1β-induced S100B secretion in three different *in vitro* preparations: cortical primary astrocytes, C6 glioma cells and hippocampal slices of rats; this secretion was mediated by MAPK-ERK/NF-κB signalling. Primary astrocytes and C6 cells exhibited different sensitivities to IL-1β concerning S100B expression and secretion. Results suggest that extracellular S100B could contribute to a reparative response in acute brain injury, as well as in neurodegenerative disorders due to chronic injury. The mechanism of IL-1β-induced S100B secretion supports the hypothesis for a "cytokine cycle", proposed in the genesis of Alzheimer disease.

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1st International Symposium about Pharmacology of Cytochrome P450

L23- A GENOMIC APPROACH TO THE ANALYSIS OF CYTOCHROME P450 REGULATION

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Pharmacogenetics has traditionally focused on variation at a single gene to explain genetic variation in drug metabolism. This is particularly true where a single enzyme is mainly responsible for the metabolism of a drug. We have taken a genomic approach to demonstrate the importance of modifier loci in influencing the expression and genetic variation of a single P450 enzyme. Caffeine is primarily metabolized by 3-demethylation to paraxanthine. In mice, greater than 80% of this 3-demethylation occurs via cytochrome P450 CYP1A2 activity. Using caffeine 3-demethylation as a quantitative phenotype, we have established that this trait is correlated to CYP1A2 activity but behaves as a complex trait in genetic analyses. A genome-wide scan was conducted for quantitative trait loci contributing to this phenotype in the F2 generation of an intercross between mouse strains with low or high caffeine 3-demethylation activity. Three loci were identified, one mapped to the *Cyp1a2* locus, and two others mapping elsewhere in the genome. Each of these loci conferred a unique phenotype when isolated in congenic strains. Different strategies were applied to identify the mechanisms by which each of these loci influenced the expression of CYP1A2 and caffeine 3-demethylation.

L24- PHARMACOGENETICS OF DRUG METABOLIZING ENZYMES ALTERS SMOKING CESSATION

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"Pharmacogenetics" is the study of inherited differences in response to drugs; we have applied this to the study of smoking behaviours and smoking cessation. CYP2A6 metabolically inactivates ≈90% of nicotine to cotinine and ≈100% of cotinine to 3OH-Cotinine. *CYP2A6* is highly genetically variable resulting in substantial differences in rates of metabolism. The ratio of 3'-hydroxycotinine (3-HC) to cotinine, derived from cigarette smoking, provides a reliable measure of nicotine metabolism rate. This presentation will review results from two clinical trials that have examined whether the *CYP2A6, CYP2B6* genotype and the nicotine metabolite ratio predicts smoking cessation and response to pharmacotherapy. In the first trial, participants (n=480) were randomly assigned to receive eight weeks of either transdermal nicotine or nicotine nasal spray (open label). The odds of quitting with transdermal nicotine were reduced by 30% with each increasing quartile of the nicotine metabolite ratio (OR=0.72, C.I.=0.57-0.90, p=0.005). That is, faster metabolizers of nicotine were less successful in quitting than slow metabolizers. The ratio did not predict cessation in the nicotine spray group, in part, because participants were able to titrate dosing to accommodate their different rates of metabolism. To identify an efficacious alternative therapy for smokers with faster nicotine metabolite ratios who performed poorly using transdermal nicotine, we examined the predictive validity of the nicotine metabolite ratio in a placebo-controlled randomized clinical trial of bupropion. Results from this trial (n=414) showed a significant interaction between the continuous nicotine metabolite ratio and treatment (p=0.04). Among smokers in the 4th quartile of nicotine metabolite ratio (fastest metabolizers), there was significant effect of bupropion therapy at the end of treatment (quit rates of 10% vs. 34% for placebo and bupropion, respectively; OR=4.56, C.I.=1.54-13.53; p=0.006) and 6-month follow-up (quit rates of 8% and 27%, respectively; OR=3.93, C.I.=1.20-12.92; p=0.02). In this second

study we also found that *CYP2B6* genotype was associated with bupropion vs placebo effects. There was a significant *CYP2B6* genotype by treatment interaction at the end of treatment (OR=2.97, C.I.=1.05–8.40, p=0.04), which was similar at 6-month follow-up (OR = 2.98, C.I.=.98–9.06, p=0.05). Among smokers in the *CYP2B6*6* group (*CYP2B6*1/ *6 and CYP2B6*6/ *6* genotype, n=147, 45% of the population), bupropion produced significantly higher abstinence rates than placebo at the end of treatment (32.5% vs. 14.3%, p=0.01) and at the 6-month follow-up (31.2% vs. 12.9%, p=0.008). In contrast, bupropion was no more effective than placebo for smokers in the *CYP2B6*1* group (*CYP2B6*1/ *1*, n=179) at the end of treatment (31.0% vs. 31.6%, p = .93) or at the 6-month follow-up (22.0% vs. 21.5%, p=0.94). For *CYP2B6*, the *CYP2B6*1* normal metabolizers (55% of population) did exceptionally well on placebo, receiving no additional benefit from bupropion. In contrast *CYP2B6*6* slow bupropion metabolizers did very well on bupropion versus placebo, maintaining the high abstinence rates at 6 months post treatment. These approaches improve our understanding of how genetically variable drug metabolism can alter smoking cessation outcomes. It suggests that a pretreatment blood test to determine smokers' nicotine metabolism rate or genotype might be useful in screening smokers to determine likely success with the two most widely used forms of pharmacotherapy. This was supported by CAMH, CIHR-MOP86471, NIH DA020830, NIDA CAP5084718 & NCI 63562 grants and a Canadian Research Chair in Pharmacogenetics. RFT is a shareholder in Nicogen Research Inc, a company focused on the development of smoking cessation treatments.

L25- EFFECTS AND CONSEQUENCES OF CYTOCHROME P450 POLYMORPHISMS ON THE TREATMENT OF MAJOR DEPRESSIVE DISORDER

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Many antidepressants are metabolized through cytochrome P4502D6, and several also inhibit its activity. Other isoenzymes of P450 cytochromes, such as CYP2C9, CYP2C19 and CYP3A4 are also involved, however, the impact of these isoenzymes in general is less than that of CYP2D6. It is well known that there are great interindividual variations in the capacity to metabolize drugs. Between 7% to 10% of Caucasians are poor metabolizers of CYP2D6 while approximately 5% are ultra rapid metabolizers. These differences in the metabolism of certain antidepressants, can impact their therapeutic effect and their adverse drug reactions. For example, if the parent drug is more active than any metabolites produced via CYP2D6, then at therapeutic doses poor metabolizers of CYP2D6 will have significantly higher plasma concentrations than normal metabolizers and thus potentially more side effects in those patients. In contrast, ultra rapid metabolizers could have less therapeutic effects. Antidepressants vary in their affinity for the CYP2D6 enzyme thus some of them (i.e. paroxetine) will be affected more than others (e.g. citalopram). Another important consequence of the polymorphism of CYP2D6 is related to the potential for drug interactions with other central nervous system medications. Thus, when another psychotropic medication is prescribed to a patient receiving an antidepressant that is metabolized through CYP2D6 the clinician should consider various factors to evaluate the potential for interactions, including among others: a) the nature of each drugs' activity at an enzyme site (substrate, inhibitor or inducer); b) the potency estimations for the inhibitor/inducer; c) the extent of metabolism of the substrate through this enzyme versus alternative metabolic routes. Potent inhibitors of the CYP2D6 such as paroxetine and fluoxetine have the potential to increase the plasma concentrations of antipsychotic medications (e.g., haloperidol) in patients who are extensive metabolizers of the enzyme. Recognizing the possible influences and consequences of CYP450 cytochrome activity is of important clinical significance when treating patients with major depressive disorder.

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L26- PHARMACOGENETICS IN HISPANIC POPULATIONS: INTERETNIC VARIABILITY ON CYTOCHROME P450

Llerena A[1], Dorado P[1], Peñas-LLedó EM[1], Pacheco-Puig R[1], Pérez B[2], Álvarez M[2], González I[2], Ramírez R[3], Lares I[4], Sosa M[4], Alanis RE[4].

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One of the major determinants of the interindividual and interethnic variability of pharmacokinetics and drug response is the genetic polymorphism of the cytochrome P450-system enzymes (CYP). The pharmacogenetics of the CYPs in Hispanic populations, a large group of world population, including people living in Spanish speaking countries of the Americas as well as those categorized as Hispanics in the United States.
The diversity of these peoples by their country of origin or residence, culture, as well as genetic composition, the latter resulting from centuries of inter-ethnic crosses between Amerindians, Europeans and Africans. This diversity is reflected in the frequency distribution of polymorphisms at the *CYP* genes that encode the main CYP enzymes involved in the biotransformation of xenobiotics, namely CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and CYP3A5.
Different population studies reported variability in the frequency of cytochrome P450 genes among different ethnic groups. Among CYPs, CYP2D6 and CYP2C9 are implicated in the metabolism of very important drugs (antiarrihmics, antipsychotics, antidepressants, warfarin, etc). Regarding one of the most studied cytochrome P450, CYP2D6 two phenotypes have been described: subjects who have an inherited decreased capacity to metabolise drugs (hydroxylate debrisoquine) are classified as "poor metabolizers" (PM), while the rest are "extensive metabolizers" (EM). Some mutated alleles causing enzyme deficiency have been described. The existence of very rapid hydroxylation of debrisoquine due to an inherited amplification of an active gene has also been reported. Thus, interethnic differences in debrisoquine hydroxylation polymorphism and *CYP2D6* and other plymorphic CYPs genes might be responsible, at least in part, for the variations in drug disposition between ethnic groups. The variability in PMs frequencies seems to be very little between Caucasian populations, among Spaniards is around 5-7%. Oriental populations, however, differ from Caucasians.
We previously reported that the relationship phenotype/genotype for CYP2D6 and CYP2E1 is different in Nicaraguans compared to Spaniards. Also, debrisoquine hydroxylation phenotype metabolic ratio and dextrometorphan (CYP2D6) and diclofenac metabolic ratio (CYP2C9) has been studied in a Cubans. Interethnic differences in Hispanics have been found. The frequency of CYP2D6 PMs was 5.6% in Nicaraguans (NAs), 5.3% in Cuban-Caucasians (CCs), 4.9% in Spanish-Caucasians (SCs) and 4.7% in Mexican-Menonitas (MMs), respectively. This proportion was higher than in Cuban-Mestizos (CMs, 3.9%) and in Mexican-Tepehuanos (MTs, 0%). Moreover, the median debrisoquine metabolic ratio (CYP2D6 hydroxylation capacity) among EMs was higher ($p<0.001$) in NAs than in CCs, CMs and SCs. Additionally in Spaniards and Cubans, personality differences were found in relationship to CYP2D6 metabolic capacity.
In several Latin American countries, including many Amerindian groups, no information are available on any pharmacogenetic targets. With the purpose of fulfilling this information gap and to promote collaborative pharmacogenetic/genomic research in Spanish- and Portuguese-speaking peoples in the Americas and the Iberian peninsula, a network – the Iberian American Network of Pharmacogenetics and Pharmacogenomics - was recently created (www.ribef.org). This initiative represents a promising step towards the inclusion of Latin American populations among those who will benefit from the implementation of pharmacogenetic principles and tools in drug therapy.
The influence of environmental as well as endogenous factors might have influence in each location, thus clinical trials might be necessary in each location in order to recommend the right dose to each individual in each country. There are interindividual and interethnic differences in drug response, thus drug recommended doses must be adapted to every population and to every subject. **Acknowledgments and Financial Support**: These studies were supported in Spain by the Ministries of Education and Science (SAF2006/13589) and Health,

Instituto de Salud Carlos III-FIS and European Union (FEDER) grants: CIBERSAM, PI06/1681 and CP/06/0030 (PD). The study was coordinated in the networks Red Iberoamericana de Farmacogenética y Farmacogenómica RIBEF (CYTED206RT0290)._

L27- CYP2C9 GENETIC POLYMORPHISM IN A CUBAN POPULATION

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Introduction: The cytocrome P4502C9 (CYP2C9) is a polymorphic enzyme involved in the biotransformation of several commonly used drugs. The most studied allelic variants, besides the wild-type allele *CYP2C9*1,* are **2* and **3,* which are related to an enzyme with altered catalytic activity. The aim of the present study was to analyze the relationship between *CYP2C9* genotype and diclofenac metabolism in a sample of Cubans. **Methods:** 217 unrelated Cuban healthy volunteers were included for testing. For each patient urinary concentrations of diclofenac (dkl) and its metabolites (4-OHdkl, 3-OHdkl and 5-OHdkl) were analyzed by high performance liquid chromatography method to determine in urine the metabolic ratio (MR): dkl concentration/dkl metabolites concentration. The genotyping was performed using polymerase chain reaction/restriction fragment length. **Results:** The most frequently allele found was CYP2C9*1. Twelve subjects (5.5%) were homozygous or heterozygous for allelic variants *2 and *3. There was wide inter-individual variability in the values of MR. 4-OHdkl RM average for CYP2C9 *1/*1 (n=134), *1/*2 (n=41), *1/*3 (n=29) and *2/*3 (n=10) ranged closely between 0.70 to 0.77. There were no significant differences (U Mann-Whitney), between CYP2C9*1/*1 (the major values of MR) and the other studied variants. One subject showed a high value of 5-OHdkl MR as compared with the others. **Conclusions:** These present results show the relevance of pharmacogenetic studies in order to explain the differences in drug response. Moreover, they express the need to investigate the factors that can influence in the metabolic capacity of the CYP2C9 in the Cuban population.

L28- GENETIC POLYMORPHISM OF CYTOCHROME P450 2D6, 2C9, 2C19 AND GLYCOPROTEIN P IN CUBAN POPULATION

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Interethnic genetic differences in the polymorphisms of drug-metabolizing enzymes and drug transporters must be considered within the perspective of individualized pharmacotherapy. Polymorphic cytochrome P450 isoenzymes (CYPs) 2C9, 2C19 and 2D6 metabolize many drugs, also the polymorphisms of ABC-transporter p-glycoprotein (MDR1 gene) shows a great interethnic variability. Frequencies for the major CYP2C9, CYP2C19 and CYP2D6 mutated alleles and the variant C3435T for p-glycoprotein were evaluated in 140 Cuban unrelated healthy volunteers (73 males and 67 females). Genotyping was performed on peripheral leukocytes DNA by PCR-RFLP method. Genotype frequencies were in Hardy–Weinberg equilibrium. Results showed: 101 subjects (72.1 %) expressed CYP2C9*1/*1, 22 (15.7 %) expressed CYP2C9*1/*2 genotypic and 3 (2.1 %) presented the mutated allele CYP2C9*2/*2 (poor metabolisers). The rest were heterozygous for CYP2C9*1/*3 (8.6 %), no CYP2C9*3/*3 was found. For CYP2C19, 137 volunteers expressed the CYP2C19*1 (75 % homozygous and 22.3 % heterozygous), two subjects were homozygous for CYP2C19*2/*2 (2.1%) (poor metabolisers), no CYP2C19*3 was detected. Genotypes more represented for CYP2D6 were: *1/*1 (59.3 %), CYP2D6*1/*10 (20.7

%) and CYP2D6*1/*17 (10.7 %). CYP2D6 *10/*10 and CYP2D6*17/*17 were found in three volunteers (1.4 and 2.1 % respectively, poor metabolisers). Frequency of apparition of CYP2D6*3 was low, one subject expressed *1/*3 and other *3/*10. Frequencies of the allelic variants of these CYPs in Cubans were similar to those of Spanish and Africans; a high frequency of CYP2D6*17 highly present in Africans but not in Caucasians. For CYP2C9 and CYP2C19, the allelic distribution was similar to Caucasians, especially CYP2C9*3 were not found in the frequencies reported in Spanish or Africans. The frequency for C3435T variant of MDR1 gene in Cubans healthy volunteers was similar to Latin America population. Results fit to Cuban population origins.

PL07- MODULATION BY CUBAN NATURAL HEALTH PRODUCTS ON P450 ENZYMES: POTENTIAL INTERACTIONS WITH THERAPEUTIC AGENTS

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Cytochrome P450 (CYPs) are the most important enzyme system involved in the biotransformation of several xenobiotics as well as many endogenous substrates in the living organisms. P450 enzymes are responsible of the Phase I metabolism of many drugs and herbals. Today, many herbs used in the traditional medicine and compounds isolated from its have been identified as substrates, inhibitors or inducer of different cytochromes in humans. This work reports the changes on P450 system after exposure of rat hepatocytes and human microsomes to herbs products used during several years in the traditional medicine in Cuba [Mangifera indica L (MSBE), Thalassia testudinum (T.t), Erythroxylum minutifolium and confusum extracts] and mangiferin, main bioactive polyphenol in MSBE. Seven specific P450 activities were evaluated after 48 h hepatocytes exposure. MSBE reduced the CYP1A2 activity (IC_{50}=190 ug/mL), no changes were induced into the other isoforms. Mangiferin produced reductions in five P450 activities: IC_{50} values of 132, 194, > 200, 151 and 137ug/mL for CYP1A2, CYP3A1, CYP2C6, SM4OH and CYP2E1, respectively. E. minutifolium, E. confusum and T.t extracts produced small reductions in SM4OH and CYP2E1 activities. All the products increased CYP2B1 activity. Other experiments were conducted in human liver microsomes. The catalytic activity of CYP1A1/2, 2D6 and 3A4 was measured using specific substrates after exposure to MSBE, T.t and mangiferin; showing decrease of CYP1A1/2 and CYP3A4 activities, but no changes of the CYP2D6 activity. It is the first report about the effects of Cuban natural health products on P450 isoforms. As these products are used concomitant with some therapeutic agents by humans, potential interactions among them were also analyzed; suggesting that their intake may lead to herbal-drug interactions with clinical consequences.

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PLENARY LECTURES (PL) OR LECTURES (L)
MONDAY, April 21

PL08- STATUS FOR DEVELOPMENT OF UNIVERSAL VACCINES AGAINST MENINGOCOCCAL DISEASE

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For fighting and controlling the devastating meningococcal disease, it is only vaccines that provide hope for a significant public health impact and relief of parents' fear. Circulating antibodies against the capsular polysaccharide has been a well proven strategy for the important serogroups as A, C, Y, W-135 (and X). Recently the principle of making effective protein-polysaccharide conjugate vaccines for these serogroups has been shown successful. Thus, providing the option of having an effective vaccine (even for infants), that will be boostable and that offers protection of longer duration. However, for fighting serogroup B meningococcal disease the most promising options are to use sub-capsular antigens as the immunogenic target. A number of universal, cross-reactive antigens have been identified through "reverse vaccinology" and successfully tested as recombinant protein vaccines. A number of issues still warrant further investigation. Some of these are sufficient immunogenicity against all relevant epitopes (also in infants), the duration of protection and the optimal timing for a booster dose. The degree of cross-protection or the true "universality", of the vaccine formulations in various global situations is also yet an unsettled case. For evaluation of vaccine formulations relying on cross-reactive proteins, selection of strains for representation of the global epidemiological situation will be of outmost importance. Defining criteria for establishing and revising such strain-collections is currently ongoing and will be a key element in developing and evaluating new protein based vaccines in the time to come.

PL09- PROPOSAL OF A GUANINE-BASED PURINERGIC SYSTEM IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

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Guanine-based purines have been traditionally studied as modulators of intracellular processes, mainly G-protein activity. However, they also exert several extracellular effects not related to G proteins, including modulation of glutamatergic activity, trophic effects on neural cells, and behavioral effects. We propose a specific guanine-based purinergic system in addition to the well-characterized adenine-based purinergic system. Current evidence suggest that guaninebased purines modulate glutamatergic parameters, such as glutamate uptake by astrocytes and synaptic vesicles, seizures induced by glutamatergic agents, response to ischemia and excitotoxicity, and are able to affect learning, memory and anxiety. Additionally, guanine-based purines have important trophic functions affecting the development, structure, or maintenance of neural cells. Although studies addressing the mechanism of action (receptors and second messenger systems) of guanine-based purines are still insufficient, these findings point to the guanine-based purines (nucleotides and guanosine) as potential new targets for neuroprotection and neuromodulation.

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PL10- MODELING THE INTERACTION BETWEEN THE ANTI- EFFR MONOCLONAL ANTIBODY NIMOTUZUMAB AND ITS TARGET, TH EPIDERMAL GROWTH FACTOR RECEPTOR

Talavera A[1], Friemann R[1], Martínez-Fleites C[3], Rabasa A[1], Garrido G[1], López-Requena A[1], Krengel U[2], Moreno E[1].

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During the last 10 years, the epidermal growth factor receptor (EGFR) has become a widely explored target for anticancer therapy. Overexpression of EGFR occurs in malignant tumors of epithelial origin and is especially common in glioma, breast, head and neck, colon and lung cancer. Modulation of EGFR-mediated signalling is therefore an attractive therapeutic strategy. Nimotuzumab is a humanized antibody that targets the EGFR, which has shown positive results in phase II/III clinical trials in head-and-neck and brain tumors. The present work is part of a wider project aimed to elucidate the molecular mechanisms of action of Nimotuzumab. Here we report the structure of the Fab fragment of this monoclonal antibody, determined by X-ray crystallography. The immunoglobulin structural scaffold is well conserved in general, but Nimotuzumab shows quite unique structural features in its variable region. An important goal of our work was to map the epitope recognized by the antibody on the EGF receptor. For this purpose, binding studies were performed by ELISA, FACS and Biacore. Competition experiments with Cetuximab – another anti-EGFR antibody for which the exact binding epitope is known – were also carried out. Finally, we performed protein-protein docking simulations and constructed a computational model of the antibody-EGFR complex, by integrating the results from the simulations with all the available experimental binding data and structural information. As result, we obtained a coherent model at the atomic level of the interacion beween Nimotuzumab and the EGF receptor.

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1st International Workshop of ImmunoPharmacology

L29- TOWARDS ELIMINATING THE USE OF ANIMALS FOR REGULATORY REQUIRED VACCINE QUALITY CONTROL

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Traditionally, regulatory required vaccine quality control has relied heavily on the use of laboratory animals; both in animal numbers and in levels of pain and distress. Apart from animal welfare concerns, the development of 3R alternatives is also triggered for scientific and economic reasons. The relevance and reliability of some animal models has been questioned and animal studies are considered time consuming and costly. In this presentation an overview will be provided of the 3R activities that have been performed at the Netherlands Vaccine Institute (NVI). These include the evaluation of humane endpoints, the replacement of challenge procedures by serology in D, T and wP vaccine potency testing, the reduction of animal numbers using single dose potency studies instead of multi-dose studies and the development of *in vitro* alternatives to D vaccine safety testing and acP vaccine Histamine sensitisation (HS) testing. Information will also be given on the interlaboratory validation of some of the 3R models. These developments will be placed in the context of European activities such as performed by the European Centre for the Validation of Alternative Methods (ECVAM) and by the European Pharmacopoeia. Although these approaches have reduced and refined animal use in vaccine quality control significantly, numbers are still extensive and further progress can only be achieved by a fundamental paradigm shift in the concept of batch release testing. Central in progress towards elimination of animal use in vaccine quality control is the acceptance of the consistency approach. The redline in the consistency approach is that a new batch of vaccine is no longer seen as a unique product but as only one of a series of batches produced from the same seed lot. As a consequence, a batch of vaccine produced shares many of the characteristics of the previous batches that were produced from the same seed lot. This allows for a new strategy of vaccine quality control, giving emphasis to aspects such as in process testing, the implementation of GMP principles and to quality assurance (QA). These approaches particularly rely on non-animal test models, such as *in vitro* cell culture tests and on physicochemical and immunochemical procedures. An outline will be given of a protocol for the consistency approach and the potential and possible pitfalls of the approach will be discussed.

L30- THE REPLACEMENT OF THE *in vivo* ASSAYS FOR THE QUALITY CONTROL OF HUMAN VACCINES: UTOPIA OR REALITY?

Waeterloos G.

(Belgium)

Non available to the closing of the program edition.

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L31- VACCINE POTENCY ASSAYS: A CANADIAN REGULATORY PERSPECTIVE

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Vaccines are highly complex biological products and the risks of batch-to-batch variations are high due to the very nature of their production processes and starting materials. From the point of view of a regulatory agency, potency assays are one of the key elements to ensure, not only the quality and efficacy of each individual vaccine batch, but also the consistency of the manufacturing process. As such, these assays should be selected to provide the highest possible degree of confidence that the lots being tested do not significantly differ from the ones shown to be efficacious in clinical trials. Key issues include the selection of relevant assays with an understanding of their limitations, trending of test results, and the establishment of reference preparations that can be linked to the lots tested in the clinical trials. Additional challenges are related to the increasing requirements for reduction of animal testing, the development of novel vaccines for which all test reagents and standards are only available from the manufacturer, novel immunomodulators, combination vaccines containing multiple antigens as well as novel delivery systems. These issues and relevant examples will be discussed. In conclusion, intensification of international collaboration between national regulatory agencies is essential to meet these challenges and ensure access to high quality, safe and effective vaccines to all.

L32- DEVELOPMENT OF ALTERNATIVES TO PERTUSSIS VACCINE CONTROL TESTS

Dorothy Xing

(UK)

Non available to the closing of the program edition.

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L33- A NEW ALTERNATIVE METHOD FOR DETERMINING BIOLOGICAL ACTIVITY IN VACCINES CONTAINING HEPATITIS B SURFACE ANTIGEN

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Although some manufacturers have developed *in vitro* potency tests, the *in vivo* test remains as the most suitable for the evaluation of Hepatitis B component in combined vaccines. Nonetheless, the Cuban National Control Laboratory has evaluated an in-house method that allows getting reliable Hepatitis B results in vaccine combinations. The aim of this paper was to evaluate the potential interferences of the rest of components on Hepatitis B surface antigen and set up an *in vitro* potency test for lot release of combined vaccines. For that we evaluated by an inhibition ELISA some combined vaccines from different manufacturers and compared the results regarding monovalent vaccines. At the same time, we prepared and evaluated some experimental formulations in order to identify the interference source. It was shown that there is no significant interference on Hepatitis B in combined vaccines, although the in vitro results were consistently lower than monovalent vaccines. This most likely arises from a complex antigen mimicking effect of *Bordetella pertussis* whole cells conforming combined vaccines, as suggested by our evidences. In spite of this, all combined vaccines successfully passed the specification defined for our in vitro test. Besides, we got a significant correlation between the *in vivo* and *in vitro* tests. Hence, we have available a reliable, fast and accurate test for lot release of Hepatitis B in combinations, a very important results taking into account the discontinuation of Auszyme kit for 2008, the most used commercial kit for Hepatitis B *in vitro* potency worldwide.

L34- ROLE OF IMMUNOEPIDEMIOLOGY IN THE DESIGN OF VACCINATION STRATEGIES

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Introduction: Inmunoepidemiology is not a simple union between Immunology and Epidemiology, the first one directed to study the immune system and the second one focused on the populations rather than the individuals. Inmunoepidemiology investigates the influence of the population immunity on different epidemiological patterns. The evaluation of immunity against tetanus and diphtheria was used as a model to prove the role of Immunoepidemiological studies in the design of vaccination strategies. **Materials and methods:** Tetanus and diphtheria antitoxin levels were evaluated by ELISA in: 392 volunteers of all age groups in Alquizar Municipality, 96 mother-newborn pairs in Havana City, and 1356 Cuban children among 1-5 years of age. **Results:** Protective levels of tetanus antitoxin (≥0.1 UI/mL) were found in 98.47% of Alquizar Municipality volunteers. However, non-protective diphtheria antitoxin levels were detected in about 50% of adults; therefore the risk of reemergence of diphtheria is possible. Suitable levels of tetanus antitoxin were found in all mothers and their newborns. Non-protective levels of diphtheria antitoxin in 54.17% and 45.83% of mothers and newborns, respectively. After the last vaccination all Cuban children had protective tetanus antitoxin levels. Non-protection against diphtheria was detected only in 2.38% of children. **Conclusions:** 1) The vaccination strategy aimed to tetanus prevention is suitable. 2) Cuban adults are not protected against diphtheria. For that reason, boosters with tetanus toxoid should be substituted by tetanus and diphtheria toxoid vaccine intended for adolescents and adults. 3) The study of the population immunity is necessary in the design of vaccination strategies.

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L35- MECHANISM OF ACTION OF A GM3 GANGLIOSIDE NANOPARTICULATED VACCINE IN A PREVENTIVE MELANOMA PRECLINICAL MODEL

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Preventive immunotherapy is an attractive strategy for patients at a high risk of having cancer. We have previously shown that preventive immunization of C57BL/6 mice with a GM3 ganglioside nanoparticulated vaccine composed of very small size proteoliposomes (VSSP/GM3) consistently elicited the rejection of B16 melanoma cells. However the precise mechanisms by which this vaccine confers tumor protection were unknown. C57BL/6 mice were vaccinated with VSSP/GM3 emulsified with Montanide ISA 51 at biweekly intervals. B16 melanoma cells were inoculated 21 days after the last immunization. The anti GM3 antibody response was examined by ELISA. To determine the lymphocyte populations involved in tumor protection, *in vivo* depletion experiments were assessed using depleting MAbs. To verify that T cells could be primed to produce IFN γ in surviving-vaccinated animals, lymphocytes were purified from spleens and stimulated *in vitro* with B16 melanoma antigens. IFN γ release was measured by ELISA kit. We have found that induction of anti-GM3 IgG antibodies correlated with tumor protection. Surprisingly, CD8+ T cells, but not NK1.1+ cells, are required in the effector phase of the antitumor immune response. The depletion of CD4+ T cells during immunization phase did not affect the anti-tumor activity. In addition, T cells from surviving-immunized animals secreted IFNγ when were co-cultured with IFNα-treated B16 melanoma cells and DC pulsed with melanoma extract. Surprisingly, in spite of the glycolipidic nature of this antigen, these findings demonstrate the direct involvement of the cellular immune response in the anti-tumor protection induced by a ganglioside-based vaccine.

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5th International Workshop of Inflammation and Pain

L36- ALLERGY AND HYPERSENSITIVITY: NEW INSIGHTS AND TREATMENT OPTIONS

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Up to 50% of the population in highly industrialized countries is sensitized to aeroallergens. Immediate type allergy results from the generation of allergen-specific IgE antibodies mediating the clinical symptoms. A predominant TH_2 immune response and a lack of regulatory T-cells are the pathophysiological background of these diseases. Genetic susceptibility, the route of allergen exposure, the allergen dose and other unspecific factors influencing the immune system lead to sensitization or individual tolerance. Allergen specific immunotherapy is the only curative treatment of specific allergy by the induction of allergen specific regulatory T-cells. Highest efficacy has been demonstrated for the subcutaneous administration of allergens, the efficacy correlates with the allergen dose. To reduce side-effects, allergoids are frequently used, this are proteins with a modified three-dimensional structure, reducing IgE binding epitopes. Under evaluation is the use of recombinant proteins; in first clinical studies it has been demonstrated that a few major allergens of grass pollens, are effective in the treatment of grass pollen allergy. Hypoallergenic recombinant proteins of birch pollen, are just used in a clinical studies, the first results are very promising. The efficacy of a combination of allergen specific immunotherapy with immunostimmulatory compounds has been demonstrated; studies have been published using MPL and CpG-motifs. IgE as a target of a therapy with omalizumab has been shown effective in allergic asthma and allergic rhinitis. Follow up studies are necessary to analyze long-time effects of this kind of "unspecific immunotherapy".

L37- BIOTHERAPEUTICS AS AN OPPORTUNITY FOR SEVERE ASTHMA

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Asthma is a common chronic inflammatory disorder of the airways and it is estimated that about 300 million people are affected worldwide. The prevalence is increasing in many countries, nowadays about 10% of the adults in industrialized countries are affected at some point in their lives. In most patients asthma can be effectively controlled by suppression of the inflammation as well as treating bronchoconstriction. Nevertheless the disease cannot be controlled in all patients although the best available therapy is used. There is an absolute need for new therapeutics especially for this group of patients. Our knowledge about the pathogenesis of asthma as lead to novel targeted therapeutic approaches. Different immunomodulating drugs are currently under development for the treatment of asthma, the efficacy of some is studied in human clinical trials. The novel therapeutic approaches include molecules targeting cytokines like IL-2, IL-4, IL-5 and IL-13 by the use of cytokine receptor antagonists, fusion proteins and specific humanized antibodies. Other targets are TNF-α, intercellular adhesion molecules, transcription factor modulators and Syk kinase inhibitors for example. The humanized monoclonal antibody Omalizumab is already registered for the treatment of severe allergic asthma and the efficacy has been proven in several clinical studies. The effectiveness of the treatment with TNF-α antagonists has been demonstrated in some studies, but concerns over the safety of these biological limits the use in severe asthma. There is some evidence the efficacy of other the other molecules; further clinical studies must demonstrate the effectiveness in bigger clinical trials on well-defined patients.

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L38- MODIFIERS OF LEUKOTRIENES IN THE TREATMENT OF THE ASTHMA

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The leukotrienes are sulfhydepeptides that are of the action of the enzyme 5-lipoxygenase on the arachidonic acid coming from the metabolism of the phospholipids of the cellular membrane. The leukotriene A4 through a hydrolase's transformed into LTB4, a potent factor chemiotactic or for the action of the LTC4 synthetase in LTC4 the one which in turn is transformed in LTD4 and LTE4, for effect of a g-glutamil transpeptidase and of a dieptidase. These three compounds, LTC4, LTD4 and LTE4, constitute that before called the substance of slow reaction of the anaphylaxes (SRL-TO), and their effects are exercised through the union to denominated receivers Cys-LT1R. Once Samuelsson and their collaborators discovered the leucotrienos in 1978, this way as their synthesis and receivers, you then could develop drugs antagonistic, their synthesis (inhibitors of 5-lipoxygenase as the zileuton and antagonistic of the protein activators of 5-lipoxygenase, PFAP) or antagonistic of the receiving Cys-LT1R. (Zafirlukast, Pranlukast, Montelukast), which have passed to be part of the available therapeutic arsenal for the handling and control of inflammatory and allergic illnesses, especially the bronchial asthma. In the patients with persistent asthma, the leukotrienes mediate a component clinically significant of the inflammation and obstruction of the air roads. Three types of clinical asthma exist where the participation pathogenesis of cysteinil leukotrienes have been demonstrated: The allergic asthma induced by allergens, the asthma induced by aspirins and the asthma induced by the exercise. Modifiers of leukotrienes like anti-inflammatory. 1) Reduce the eosinophyls in the sputum. 2) Reduce the eosinophyls in outlying blood. 3) Reduce the eosinophyls in the bronchial biopsies. 4) Reduce the nitric oxide exhaled in asthmatic children. 5) Cellular populations of lymphocytes reduce T CD3, CD4+ and the cells EG2+. 6) They reduce the lymphocytes and basophils in the bronchial laundry obtained after bronchoprovocation with allergen. 7) They block the early and late asthmatic reaction induced by allergen and they block the bronchial hiperreactividad partially.

L39- NEW IMMUNOLOGICAL TREATMENT APPROACHES FOR ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA)

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Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disease that affects people with asthma or cystic fibrosis (CF) in the majority of cases. The disease is the result of the colonisation of the respiratory tract by Aspergillus and subsequent host sensitization to fungal antigens, accompanied by a Th2 CD4 type response mediated by the production of specific IgE. Glucocorticosteroids, the cornerstone of treatment of ABPA, have toxicities that may be especially of concern in patients with CF who are already prone to the development of diabetes, osteopenia, growth retardation and infections. The concomitant use of itraconazole seems to promote a better control of the disease and to reduce the duration of systemic steroid therapy but its use continues to be controversial. In the last decade we witnessed an enormous increase of knowledge about the pathomechanisms of ABPA and hopefully new therapeutic approaches will be generated in the future. For example, mice immunized with recombinant epitopes of Asp f1 were prevented from elaborating an immune response when they were subsequently challenged with A. fumigatus crude antigen. These results support a potential role for synthetic peptides as immunotherapeutic agents in allergic aspergillosis, but they need further study. Recently some cases of a rapid improvement of respiratory symptoms and lung function after treatment with anti-IgE antibodies (omalizumab) in CF-patients were published but clinical studies are necessary to proof the effectiveness of omalizumab in the treatment of ABPA.

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L40- RECOMBINANT INTERFERON GAMMA IN THE TREATMENT OF LIFE-THREATENING PULMONARY DISEASES

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Introduction. Tuberculosis (TB) is increasing in the world and multidrug-resistant (MDR) disease beckons new treatments. High antibiotic resistance is described in atypical mycobacteriosis, mainly by *Mycobacterium avium* complex (MAC). Idiopathic pulmonary fibrosis (IPF) is a fatal disease, where no therapy has been demonstrated to be modifier of the disease course. Interferon (IFN) gamma, a glycoprotein produced primary by CD4+ cells, plays a central role in the immunoregulatory response to mycobacterial infections through macrophages activation. Lack of production of this cytokine or expression of its receptor is associated to the infection's most lethal forms. IFN gamma has also a potent antifibrotic effect. **Materials and Methods.** Several clinical trials were conducted to evaluate the response of patients with MDR-TB, MAC infection or IPF to the treatment with IFN gamma. Patients received 1 x 10^6 IU of recombinant IFN gamma intramuscularly during 6 months, as adjuvant to specific chemotherapy. **Results.** Significant clinical, functional and imagenological improvement was observed in patients treated with IFN gamma since an earlier disappearance of respiratory symptoms and a pulmonary lesions reduction were observed. Those patients with mycobaterial infection converted rapidly the sputum samples to negative. Serum levels of profibrotic TGF-beta and advanced oxidation protein products increased significantly in the MAC placebo group but not among IFN receiving patients. Treatment with IFN gamma was good tolerated, without severe adverse events, mostly related with the well-know induced flu-like syndrome. **Conclusion.** These data suggest that IFN gamma is useful and safe as adjunctive therapy in patients with life-threatening pulmonary diseases.

L41- REVIEW ON THE ANTI-SARS WORKS IN HOSPITALS IN CHINA

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Objective: To review the Anti-SARS works of Chinese Hospitals and to discuss the medical emergency system of dealing with massive critical events in the hospitals. **Methods:** From the results obtained through the experimentation of dealing with each individual case, an emergency system with appropriate regulations is to be established so as to control and restrain the sources of disease transmission, to protect the high risk population such as medical staffs. **Results:** A Series of measurements were taken during this process, which are later on proved to be effective. SARS infection was put under control eventually. The SARS infection of the workers and staff of clinical and health care departments, hospitals in China were successfully prevented. **Conclusions:** Emergency mechanism and system for monitoring and controlling the epidemic in early stage should be set up in hospitals. Infectious disease clinics and inward isolating area should be built for the strike of emergency public health events. Chinese traditional medicine played an important role in the massive counterattack to the SARS virus. Herbs greatly helped the increase of immune system of human body.

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L42- STUDIES OF CUPRIZONE INDUCED NEUROINFLAMMATION

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Multiple sclerosis (MS) is a common chronic inflammatory demyelinating disorder of the central nervous system (CNS). The disease is complex and its aetiology is multifactorial and largely unknown. In deciphering the pathological mechanisms behind multiple sclerosis (MS), the study of experimental autoimmune encephalomyelitis (EAE) has laid the foundations for gaining a more comprehensive understanding of the autoimmune response which presumably is, if not the cause of the disease, an essential component in the perpetuation of the pathological process. However, one of the limitations of employing EAE as an animal model for MS is that the demyelination, and in particular remyelination occurring in response to the autoimmune attack is limited. In fact, one of the most frustrating aspects associated with the human disease is the inadequacy healing response (remyelination). Here, we examined the role that the several genes might play in the processes of demyelination and remyelination by using an animal model of toxic demyelination (cuprizone-induced model of demyelination/remyelination). In conclusion, our studies highlight the importance of the use of various animal models for evaluating the contribution of particular genes to complexity of the human disease.

L43- A DEMYELINATING MODEL INDUCED BY CUPRIZONE. INFLUENCE OF TRANSFERRIN AND THYROID HORMONES

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Wistar rats were fed a diet containing 0.6% cuprizone (CPZ) for two weeks to induce demyelination. After CPZ withdrawal at 35 days of age, rats were injected either intracranielly with 350ng apotransferrin (aTf) or subcutaneously on day 35, 37 and 39 with T3. Biochemical studies of isolated myelin from CPZ-treated rats, showed a marked decrease in proteins, phospholipids and galactolipids as well as a marked decrease in myelin yield. Histological and immunohistochemical analysis also showed an hypomyelination in the experimental animals. Treatment of these animals with a single intracranial injection of aTf induced a marked increase in myelin deposition resulting in a significantly improved remyelination, evaluated by histological, immuno-histochemical and biochemical parameters. In comparison with to what was observed in spontaneous recovery. With reference to T3 treatment ,histological studies showed an increased remyelination in the corpus callosum (CC) of T3 treated animals when compared with to control one. These results suggest that after CPZ-induced demyelination, thyroid hormone regulates central nervous system (CNS) remyelination process by inducing the differentiation of the progenitor cells of the SVZ and accelerating the oligodendroglial maturation in the corpus callosum.

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L44- THE CONTRIBUTION OF NITRIC OXIDE AND INTERFERON GAMMA TO THE REGULATION OF THE NEURO-INFLAMMATION OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Nitric oxide (NO) is a key messenger involved in physiological functions including endothelium-dependent vascular relaxation, inhibition of platelet adhesion and aggregation and regulation of inflammatory and immune responses. I will briefly introduce NO and its functions and then describe our work over the past several years examining the role of NO in both rat and mouse experimental autoimmune encephalomyelitis (EAE). Furthermore since interferon gamma (IFN□) is one of the prime stimulators of NO production by many cell types I will present data linking the regulatory role that IFN□ has on EAE via NO production

L45- REPEATED PRO-INFLAMMATORY DEMYELINATING STIMULI MAY RESULT IN REDUCED INFLAMMATION AND DEMYELINATION

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Patients in the relapsing phase of MS show high levels of IL-1β in the cerebrospinal fluid, and blockade of this cytokine reduces the severity of the disease. We have previously observed that chronic expression of IL-1β in the striatum produces reversible demyelination, blood-brain barrier breakdown and axonal damage, but no neurodegeneration. Most MS patients present relapsing-remitting episodes, and considering that remyelination is less efficient after every relapse, our hypothesis is that repeated pro-inflammatory stimulus in the brain could exacerbate demyelination in animals which had received a previous demyelinating stimulus. To contrast this hypothesis, we administered either adenoviral vectors expressing IL-1β (AdIL-1β) or unrelated inflammatory stimuli (LPS or PolyI:C). At different time points, we re-administered the AdIL-1 β in the striatum. We used β-Galactosidase (Adβ-gal) as a control of the AdIL-1β. Animals receiving pro-inflammatory stimuli in both opportunities had less inflammation and demyelination than animals receiving a unique pro-inflammatory stimulus. Additionally, a difference in the quality of the infiltrate was observed: animals which received repeated pro-inflammatory stimuli had a higher proportion of neutrophils. To asses the response of the tissue in different stages of the remyelinating process, we administered the second pro-inflammatory stimuli after 51 days. With this protocol, we observed less inflammation and demyelination than the earlier stimulus. Finally, we observed that the astrocytic and microglial activation followed the same pattern of the inflammation. In conclusion, contrary to our hypothesis we observed that a previous pro-inflammatory stimulus may reduce the inflammatory and demyelinating effect of a second pro-inflammatory stimulus, when administered in a 30 days interval.

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L46- NEUROIMMUNOREGULATION, A TOOL FOR THERAPY IN DEMYELINATING DISEASES

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Some evidences support the autoimmune etiology of demyelinating disease, multiple sclerosis and NMO. Both of them, show that arms innate and adaptative of the immune response are involved in the aberrant mechanism against antigens associated to myelin, axonal and neuronal damage, once immune activation have occurred. Various strategies have been designed conferring protection or preventing damage in demyelinating diseases, nevertheless a no real efficacy may be delimited. Strategies to prevent these damages involve from a deviation of Th1 to Th2 cell pattern, functional inhibition of metalloproteinase activity, apoptosis inhibition and antibody intervention into other as well as differential efficacy has been obtained in experimental models and human. The following presentation will present results of reports in human and animal models and review the most recent concept on the immune-intervention in the modulation of these pathogenic events, focused to the antibodies as a tool for therapy in these diseases

L47- T CELL IMMUNOREGULATION AND OXIDATIVE STRESS IN NEUROMYELISTIS OPTICA

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Introduction: Neuromyelitis optica (NMO) is an idiopathic central nervous system (CNS) demyelinating syndrome although the cause of the disorder is not known; several lines of evidence suggest that the fundamental immunological process is driven by humoral mechanisms. Clinical experience suggests that plasmapheresis and immunosuppressive therapies are beneficial for treatment and prevention of acute attacks however the standard immunomodulatory drugs may not alter the course of NMO suggesting the involvement of other factors in the NMO pathogenesis. Conversely to humoral immune arm, cytokines, chemokines related to the cellular immune response, the blood barrier integrity markers and oxidative stress component have been poorly studied. **Materials and methods:** We performed a molecular characterization of cellular immune response and oxidative stress parameters in serum from NMO patients. We measured the serum levels of TNF-α, IFN-γ, IL-10, MMP-9 metalloproteinase, its inhibitor TIMP-1 and oxidative stress markers: malondialdehyde, advanced oxidation protein products, peroxidation potential, superoxide dismutase, catalase, and total hydroperoxides from NMO patients and healthy controls. **Results:** Serum levels of TNF-α and IL-10 cytokines from NMO patients were down regulated suggesting that in addition to a humoral disregulation, an effector - regulator imbalance could be implied in its pathogenesis. Furthermore, we detected an up regulation statistically significant of all parameters of oxidative stress studied indicative of a therapy antioxidant is required. **Conclusion:** NMO is characterized by a loss of the T-cell regulation and noteworthy redox imbalance.

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L48- THERAPEUTIC INTERVENTIONS IN THE TREATMENT OF MULTIPLE SCLEROSIS

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Multiple Sclerosis is a demyelinating disease of the CNS affecting close to 2 million people worldwide. Currently there are a limited numbers of drugs on the market to treat this disease, all of which are anti-inflammatory injectables. There is no current therapy for MS patients with secondary progressive disease, a population constituting 35% of all MS patients. There are no orally available drugs which are disease modifiers. There are no neuroprotective therapeutic agents which could preserve myelin, axons, oligodendrocytes, or neurons. Nor are there any drugs to drive remyelination by activating endogenous stem cells. The Neurology group at *sanofi-aventis* is approaching the therapeutic intervention of this disease by addressing all these aspects of a major unmet medical need in MS treatment. Our goal is to produce novel first in class drugs which are safe, efficacious, and easily administered, allowing for improved quality of life and fewer side effects than the current treatments. The *in vitro* and *in vivo* models for developing therapeutic agents in MS as well as the general principles of drug discovery will be discussed and exemplified.

L49- MULTIPLE SCLEROSIS IN CHILDHOOD: SCIENTIFIC EVIDENCES

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Introduction: Multiple Sclerosis (MS) has been traditionally a disease under the control of an adult neurologist. Although the descriptions of childhood appeared at the beginning of last century, it is not until the 80´s that MS in childhood was recognized and well characterized. It is of a generalized opinion, that neuropediatricians are time–resource consuming in the investigation of other diseases before diagnosing MS. **Objective:** To characterize such disease based on scientific evidence of the essential aspects of MS in childhood. **Patients and Methods:** This characterization was performed following the methodology to make clinical guidelines based on evidences from the American Academy of Neurology to characterize MS in children. For the search of scientific information, was found support in the following database: PubMed, TRIP database and Cochrane Library. The obtained information was then classified for its clinical evidence and conclusions. **Results and Conclusions:** The results of the meta analysis gave as a total of 643 patients with MS in Childhood up to December 2007. With Ia type-evidence the prevalence was accepted as to the sex distribution, the largest evolution with respect to adults with a much more frequents monosymptomatic presentation. It was also accepted with Ib type-evidence, the children have less lesion than adults according to Poser et al, diagnosis that is, criteria gave a definite diagnosis of MS. Those pertaining to Mac Donald´s, still have to demonstrate their validity during childhood. The rest of clinical, complementary and treatment characteristics demonstrated evidence levels between II and IV.

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L50- NEUROMYELITIS OPTICA SPECTRUM DISORDERS IN CUBA

Cabrera-Gómez JA.

LACTRIMS

The prevalence rate of NMO in Cuba was 0.52 per 100,000 population (95% CI 0.39-0.67), with a female rate of 0.91 (95% CI 0.68-1.20) and a male rate of 0.12 per 100,000 (95% CI 0.05-0.25) with an estimated average annual incidence rate as 0.053 per 100,000 population (95% CI 0.040-0.068). We presented the description of familial cases of R-NMO and MS in sisters and review 5 familial cases of NMO-SD. Two familial cases were compatible with m-NMO, a third report with familial R-NMO and the fourth two cases studied were in a Cuban family with R-NMO and R-LETM. We observed a higher frequency of R-NMO and r-LETM over r-LETM-SNC, m-NMO and r-ON. Black patients had a longer duration of disease, more relapses and more abnormalities in brain MRI (not meeting MS criteria) . R- LETM, has a more delayed age at onset, attains a higher physical disability with a lower number of relapses in a shorter period of time, as compared to the others forms. The predictive factors in RNMO for early death were lack of recovery from first attack and first inter attack interval < one year which predicted a shorter time from onset to death.We also described the characteristics of the brain MRI lesions in NMO for the differential diagnosis with MS using the MRI protocol of the Consortium of Multiple Sclerosis Centers. We demonstrated that the brain MRI pattern of R-NMO patients is different from MS and none of the brain MRI abnormalities not fulfilled MS criteria. NMO-IgG cases had more relapses, a high score in disability, motor and sensorial and more number of brain MRI lesions (not fulfilled MS criteria).However, cooperative studies are needed to learn the natural history and mortality of NMO in the Caribbean, since in Martinique NMO cases apparently predominated in mixed blacks, whereas in Cuba they were equally prevalent in whites, blacks, and mixed subjects. This cooperative study is in process, and it will allow a more detailed evaluation of the natural history and mortality of NMO in the Caribbean with a larger series of cases.

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1st International Symposium about Pharmacology of Cytochrome P450

L51- ROLE OF CYTOCHROME P450 MODULATION ON MUTAGEN ACTIVATION AND DRUG-DRUG INTERACTIONS

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Cytochrome P450 (CYP) denotes an enzyme superfamily localized mainly in liver but also in other tissues like brain, skin, kidney, and intestine, among others. The targets of these enzymes are constituted by endogenous and exogenous molecules including environmental mutagens and widely used drugs. The variation in expression and activity of these proteins could lead to at least two scenarios: the enhancement or inhibition of the metabolism of environmental mutagens involved in the process of carcinogenesis, and drug-drug interactions leading to medical adverse reactions. Using rodents as experimental model along with biochemical/immunological techniques and genetic toxicology methodologies, our group has been interested in the modulation of CYP activity by antiparasitic drugs like albendazole, the intake of diets with different concentration of protein or sodium chloride, and by the consumption of diet factors like grapefruit juice. Results show that all of them are able to modulate the activity and/or concentration of CYP in liver and other tissues. Albendazole increase the activity and concentration of CYP1A in liver microsomes. Obtained results indicate that the induction pathway is different to that in which cytosolic AhR receptor is involved. In comparison to the ingestion of a normal diet containing 24% protein, a low protein diet decreases the activity of liver CYP1A, CYP2B and CYP2E. Finally, high concentration of sodium chloride in diet induces the CYP1A and CYP3A immunoreactive protein detected in gastric mucosa and grapefruit juice modifies the normal CYP3A and CYP1A activity in intestine and liver. Possible risks or benefits derived from the use of CYP modulators as a strategy to face the action of environmental mutagens/carcinogens as well as the interactions between drugs metabolized by the same CYP enzymes will be discussed.

L52- REGULATION OF DRUG TRANSPORTERS IN HEALTH AND DISEASE

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While it is a well established fact that drug disposition is often altered in patient populations, the impact of underlying disease states has been largely neglected as an important source of inter-subject variability. Studies in patients and animal models over the past 20 years have demonstrated that underlying infectious or inflammatory conditions are associated with alterations in the production of numerous liver-derived proteins and altered plasma drug concentrations. Transport proteins play a critical role in the absorption, distribution and clearance for numerous drugs, toxins and their metabolic products and may thus play a role in these changes. Specifically, several of the ATP-binding cassette (ABC) transporters P-glycoprotein (PGP), the multidrug resistance associated proteins (MRPs) and the breast cancer resistant protein (BCRP) are highly expressed in biologically protective barriers of the liver, intestine, blood brain barrier and placenta and are believed to profoundly limit the passage of therapeutic or toxic xenobiotics across these membranes. While the role of ABC transporters in drug transport has been examined under normal physiological conditions, the impact of disease on the regulation and activity of these drug transporters are poorly understood. Using *in vivo* and *in vitro* models of inflammation, we have subsequently examined the role of inflammatory disease and cytokines in the regulation of transporters. Studies in our laboratory found that infection and inflammation alters the expression

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and activity of several members of the ABC family of drug efflux transporters. Studies in endotoxin and cytokine-treated rodents demonstrated significant reductions in the hepatic expression of the ABC transporters: PGP, MRP2, MRP3, MDR2 and BSEP as well as several organic anion (OATP) transporters. Expression and activity of many of these transporters were also found to be suppressed in the intestine, brain and placenta. These changes appear to occur primarily through cytokine- mediated pathways. Indeed, in both rodent and human cell lines, these changes have been found to primarily stem from an IL-6 mediated alterations in gene transcription. As activation of the Pregnane X nuclear receptor (PXR) results in the induction of many of the same transporter and drug metabolizing enzymes that were found to be suppressed during inflammation, we hypothesized that PXR could be involved in down-regulatory pathways. Indeed, *in vivo* studies in endotoxin and IL-treated PXR knockout mice demonstrated a partial role of PXR in the downregulation of both drug transporter and metabolizing enzymes, thereby revealing a novel mechanism by which these proteins are regulated. Inflammation-mediated suppression of the ABC transporters were also found to result in corresponding changes to the *in vivo* and *in situ* transport and tissue accumulation of model substrates of these transporters including doxorubicin, digoxin and the radioimaging agent ^{99m}TC-sestamibi. Specifically, endotoxin-induced downregulation of efflux transporters was associated with corresponding increases in intestinal absorption and increased drug accumulation in brain and fetal tissues of their substrates. As the ABC family of drug transporters are involved in the distribution and elimination of a large number of chemically diverse and clinically important drugs, our studies suggest that disease-induced changes in the expression and activity of these proteins likely contribute to intra- and inter-subject variability of drug disposition and response in patients.

L53- IMPACT OF INFLAMMATION ON DRUG DISPOSITION IN CANCER: REPRESSION OF HEPATIC CYP3A AND DRUG TRANSPORTER GENES BY TUMOUR-DERIVED CYTOKINES

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A major challenge in pharmacogenomics is the narrow therapeutic index of anti-cancer treatments, as toxicity due to variable drug clearance is a common cause of treatment failure. CYP3A4 is the major pathway of human drug metabolism, responsible for clearance of over half of all anti-cancer agents. While many studies have sought to establish links between CYP3A4 polymorphisms and altered pharmacokinetics of anti-cancer agents, genetic differences do not appear to account for variable CYP3A-mediated drug metabolism in cancer. Recently, we observed that reduced CYP3A4 activity in cancer patients was correlated to the degree of systemic inflammation, resulting in enhanced toxicity of anti-cancer drugs (1). To investigate hepatic expression of the human *CYP3A4* gene in the presence of cancer, we used a transgenic mouse model of the *CYP3A4* regulatory region attached to *lacZ.* The EHS sarcoma was injected into the hind limb of transgenic *CYP3A4/lacZ* mice. Markers of inflammation included cytokine levels and hepatic expression of Serum Amyloid protein P (SAP), the major mouse acute phase protein. Assessment of CYP3A function by the midazolam sleep test showed that midazolam-induced anaesthesia was increased in tumour-bearing mice. Hepatic CYP3A4 and mouse Cyp3a11 expression was decreased in tumour-bearing animals (2), while SAP expression was increased 8 fold. The mRNA expression levels of other CYPs and drug transporters including Mrp2, Mrp3, Oatp2, Oatpc, MDR2 and Bcrp was also down-regulated in the liver (3). Protein profiling by advanced MS-based techniques show that multiple CYPs, phase II enzyme and drug transporters are reduced. We have also observed reduced CYP3A and drug transporters associated with inflammation in mice with breast, melanoma and colon explant tumours showing that repression of drug clearance pathways is a feature of diverse cancers. Evidence for the involvement of IL-6 included raised serum concentrations, increased phospho-STAT3 protein, activated MAP kinases and SOCS3 mRNA levels in the liver. Blocking IL-6 action with a specific antibody partially restored Cyp3a11 levels. CONCLUSIONS This study has shown for the first time that cancer-associated inflammation transcriptionally represses drug clearance pathways. The transgenic mouse model of human CYP3A4

regulation, coupled with explant tumour models, permits pre-clinical testing of anti-inflammatory interventions aimed at making cancer treatment safer and more effective. Reduced levels of hepatic drug transporters, as opposed to enhanced expression within cancer cells, has significant implications for the management of multi-drug resistance in tumours. Consideration of the impact of inflammation associated with tumours on the pharmacokinetics of anti-cancer drugs may make a significant contribution to individualised treatment. **References: 1)** Slaviero et al. Lancet Oncol 4: 224-233, 2003. **2)** Charles et al. Clin Cancer Res 12: 7492, 2006. **3)** Sharma R, et al. Br J Cancer 98:91, 2007.

L54- CYTOCHROMES P450 AND NON-STEROIDAL ANTIINFLAMMATORY DRUGS, PREVENTION OF GASTROINTESTINAL BLEEDING

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Introduction. Acute gastrointestinal bleeding associated with the use of NSAID is a common adverse drug reaction with a high rate of hospitalization and mortality in developed countries. Known risk factors related to acute gastrointestinal bleeding related to NSAID include age, dose, and previous history of gastrointestinal diseases. In addition, increasing evidences support the hypothesis that genetic factors modulating NSAID biodisposition may modify the risk to develop NSAID-related gastrointestinal bleeding, thus raising the possibility for the use of pharmacogenomic tests to identify high-risk individuals. **Methods**. Common CYP2C8 and CYP2C9 polymorphisms were studied in a cross sectional study involving 134 NSAID-related bleeding patients and in 177 patients receiving NSAID with no adverse effects. **Results**. Among patients receiving NSAID that are CYP2C8/9 substrates the frequencies for carriers of variant alleles versus control subjects were: CYP2C8*3: 0.50 vs 0.23 (OR; 95%CI=3.4; 1.5-7.5; p=0.002), CYP2C9*2: 0.48 vs 0.26 (OR; 95%CI=2.7; 1.2-5.8; p=0.013) and CYP2C9*3: 0.24 vs 0.20 (OR; 95%CI=1.3; 0.5-3.1; p=0.578). The frequencies for carriers of the CYP2C8*3+CYP2C9*2 genotype were 0.40 vs 0.15 (OR; 95%CI=3.7;1.6-8.9; p=0.003). Among bleeding patients receiving NSAID that are not extensively metabolized by CYP2C8/9 no differences in genotypes or allele frequencies were observed as compared to control subjects. **Conclusions**. The combined presence of CYP2C8*3 and CYP2C9*2 (CYP2C8*3+CYP2C9*2 genotype), is a relevant determinant in the risk to develop gastrointestinal bleeding in patients receiving NSAID that are CYP2C8/9 substrates. **Financial support**: Grants FIS 05/1056, 06/1252 and RETICS 07/0064/0016 from Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Madrid, Spain.

L55- FROM PHARMACOGENETICS TO PERSONALIZED MEDICINE: A REGULATORY PERSPECTIVE

Remirez D

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The science of pharmacogenomics has advanced significantly in the last five years, but it is still in infancy and is mostly used on research basis. The Pharmacogenomics helps identify interindividual variabilities in drug response (both toxicity and effectiveness). This information will make it possible to individualize therapy with the intent of maximizing effectiveness and minimizing risk. The aims of this work are to present the bases of pharmacogenetic, the advantage and challenges of this specialty, the main enzymes characterized for the genetic polimorphism and the world and cuban regulatory perspective about this subject. We will show the main biomarkes for pharmacogenetics studies and a general guidance for submission of this type of research. The

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hope for the future is that through personalized medicine, doctors and patients will be able to make better-informed choices about treatment. This treatment will avoid the adverse drug reaction to the medication and will improve the diagnosis diseases as well as the prevention and treatment of diseases.

PL11- IMMUNOTOXICITY EVALUATION IN SAFETY ASSESSMENT OF DRUGS. CURRENT AND FUTURE PERSPECTIVES

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Immunotoxicology is an important aspect of the safety evaluation of drugs and chemicals. Immunotoxic effects are divided into four categories: immunosuppression, immunostimulation, hypersensitivity and autoimmunity. Even though concern primarily focused on immunosuppression, hypersensitivity and immunostimulation are also key issues, especially with pharmaceuticals. However, the nonclinical assessment of immunotoxicity is currently often restricted to animal models and assays to predict unexpected immunosuppression. There is no general consensus so that a variety of assays can be considered depending on the compound to be tested. A major issue is whether histological examination lymphoid organs is a reliable predictor of immunosuppression or whether immune function should also be assessed. A T dependent antibody response assay, either the plaque forming cell (PFC) assay or antiKLH ELISA, is recommended as a first line assay. A variety of assays, including lymphocyte subset analysis, NK cell activity assay, lymphocyte proliferation assay, delayed type hypersensitivity assay, cytotoxic T lymphocyte activity assay and macrophage/neutrophil function assays can also be used. In certain circumstances, host resistance assays can be considered. With the exception of contact sensitization, very few animal models and assays can reliably predict the potential for unspecific immunostimulation, hypersensitivity or autoimmunity. A major limitation of immunotoxicity risk assessment is the lack of human data. Immunological endpoints and clinical criteria to be included in clinical trials and epidemiological studies have to be carefully standardized and validated. In this lecture are presented the current and future perspectives for the immunotoxicological evaluation.

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PLENARY LECTURES (PL)
TUESDAY, APRIL 22

PL12- MECHANISMS OF IMMUNE MEDIATED INJURY AND REPAIR IN THE CENTRAL NERVOUS SYSTEM

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The most established primary central nervous system (CNS) immune mediated disorders, namely post-vaccination encephalomyelitis in human and experimental autoimmune encephalomyelitis in animals are initiated by systemic immunization with neural antigens and generation of autoreactive T cells. Persistence, activation, and effector responses of such cells in the CNS requires interaction with antigen presenting cells (APCs) that deliver signal related to antigen/MHC (signal 1), co-stimulatory molecules (signal 2), and polarizing cytokines. APCs in the CNS include innate immune constituents in the perivascular space (ecently arrived monocytes, dendritic cells) and in parenchyma (microglia). These functional properties of APCs can all be modulated by signals from their microenviroment, including “stranger” and “danger” signals, and by infiltrating immune cells and molecules.
Target selective Cytotoxicity is classically linked to adaptive immune mediators (B cells/immunoglobulin and $\alpha\beta$ T cells). Oligodendrocytes, neurons, and astrocytes can express MHC class 1 molecules and be targets of antigens/MHC restricted $\alpha\beta$ CD8 T cells. Such cells can also be targeted by innate immune effectors ($\gamma\delta$ T cells, NK cells) or $\alpha\beta$ T cells re-programmed by pro-inflammatory cytokines to adquire innate immune properties. Target selectivity depends on inflammation induced expression of specific ligands for the innate cell associated receptors. Fc receptor expressing innate cells can also interact with Ig to effect target selective injury, a process termed antibody depend cell cytotoxicity.
Inflammatory molecules also contribute to the tissue repair process by removing tissue debris and serving as chemattractants for progenitor cells. Thus, immune-directed therapies need consider the dual roles of neuro-inflammation.

PL13- BIOPHARMACEUTICAL APPROACH TO AUTOIMMUNE MECHANISMS IN NEUROLOGICAL DISEASE THERAPY

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The therapeutic potential of natural products from animal, plants, microorganisms or other biological sources (biopharmaceuticals) is permanently under scrutiny, in the search of inherent properties conferring them pharmacological activities such as the capacity of scavenging reactive oxygen species (ROS), interacting with immune (innate or acquired) effectors, regulating components or enzymes of the inflammatory cascade or triggering the production of cytokines, among other mechanisms of action. These biopharmaceuticals may be complex mixtures from natural sources or components extracted and more or less purified or concentrated industrially and could modify the natural course of diseases or conditions such as senility, neurodegenerative or ischemic disorders, leading to organic and functional damage of the central nervous system (CNS), which are produced or include auto-reactive immune responses linked to their pathogenic mechanism. Some of these “natural modifiers” are selectively chosen for revision and evaluated in terms of the evidence available from the literature and results of the author's working group.

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PL14- PROTEIN KINASES AS PROMISING PHARMACOLOGIC TARGETS FOR CANCER THERAPEUTICS. DEVELOPING OF CIGB-300, A NOVEL PROAPOPTOTIC SYNTHETIC PEPTIDE THAT IMPAIRS THE PROTEIN KINASE CK2-MEDIATED PHOSPHORYLATION.

Perea SE.

Center for Genetic Engineering and Biotechnology (CIGB), Havana 10600, Cuba

Exploitation of protein kinases as cancer therapeutic targets is continuously growing and its clinical validation becomes a reality as therapies with some specific inhibitors have showed clinical benefit in cancer patients. Thus, some specific tyrosine kinase inhibitors targeting the growth factor receptors have been successfully used as molecular targeted therapy to treat solid tumors. Likewise, chemicals targeting the abl-bcr fusion protein have been also developed with significant impact on Chronic Myeloid Leukemia. CK2 phosphorylation event is a promising target for cancer therapeutics as it is involved on cell malignant transformation, protection against apoptosis, metastasis, and angiogenesis. The C.I.G.B. in Cuba has developed an innovative approach to target the CK2-mediated phosphorylation using a peptide-based drug (CIGB-300) originated by a hypothesis-driven screening. Contrary to the paradigmatic strategy blocking the ATP-binding site, CIGB-300 targets the phophoaceptor site on the CK2-substrates impairing its phosphorylation by out competing the enzyme. In line with this, CIGB-300 induces apoptosis on tumor cell lines, and exhibit antitumor effect in different cancer animal models. Importantly, CIGB-300 has reached the clinical ground and a dose-escalation Phase 1 clinical trial has been already performed in patients with cervical malignancies. The peptide was safe, well tolerated and signs of clinical benefit were registered after treatment. These data has supported the further clinical develop of CIGB-300. Our results provide an early proof-of-principle of clinical benefit by using an anti-CK2 approach in cancer. Furthermore, this is the first clinical trial where an investigational drug has been used to target the CK2 phosphorylation.

PL15- GANGLIOSIDE-BASED CANCER IMMUNOTHERAPY: TWO VACCINATION APPROACHES

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Active specific immunotherapy is an emergent field which might have impact in the treatment of chronic diseases such as cancer and autoimmunity. Then therapeutic vaccines constitute a new category of biopharmaceuticals which deserve attention by the pharmacologists´ community. Vaccination is a process of connecting innate and adaptive immune systems to generate an antigen specific immune response. Nowadays, there is enough scientific information to induce an effective immune response to any protein antigen, but the immune regulation for glycolipid antigens is less known. Moreover, an additional issue in chronic diseases would be the impairment of the immune system function. During the last decade, we have been targeting NeuGcGM3 ganglioside for cancer therapy. Two different approaches have been followed: a molecular vaccine consisting in the hydrophobic incorporation of gangliosides into the Outer Membrane Proteins (OMP) from *N meningitides,* which renders nanoproteosomes, and an anti-idiotypic monoclonal antibody as antigen surrogate. The nanoproteosomes were proven to induce activation of Dendritic Cells (DC) and to be immunogenic in cancer patients. On other hand, the idiotype vaccine is also immunogenic, generating both Ag+Id+ and Ag+Id- antibodies. We hypothesize B1 cells might be involved in the generation of Ag+Id- antibodies. At present, preliminary evidences of therapeutic efficacy have been obtained for both vaccination approaches. Possible involvement of cellular immune response will be discussed.

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PL16- IMMUNOPHARMACOLOGICAL RESEARCHES IN CUBA. RESULTS AND PERSPECTIVES. ITS IMPACT IN CUBAN SYSTEM OF HEALTH

Delgado R.

(Cuba)

Non available to the closing of the program edition.

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POSTERS (P)
SUNDAY, APRIL 20

1st International Workshop of ImmunoPharmacology (IP)

Chairs: Pedro Camilo Rodriguez, Eva Marrero, Gilberto Pardo, Grisel del Toro, Maria Acelia Marrero, Beatriz Garrido (Cuba)

PIP01- CHARACTERIZATION OF A NON-APOPTOTIC CELL DEATH INDUCED BY AN ANTI-N-GLYCOLYL GM3 ANTIBODY

Roque-Navarro L[1&], Chakrabandhu K[2], de León J[1], Rodríguez S[3], Toledo C[4], Carr A[1], Mateo de Acosta C[1], Hueber AO[2] and Pérez R[1].
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Introduction: Gangliosides have been involved in multiple processes such as growth, differentiation, adhesion and more recently as regulators of cell death pathways. Some of these molecules can be considered as tumor-associated antigens, in particular, *N*-glycolyl sialic acid containing gangliosides are promising candidates for cancer targeted-therapy because of their low expression in normal human tissues. In this study, we provided a molecular and cellular characterization of a novel cell death mechanism induced by the anti-NGcGM3 14F7 mAb. **Materials And Methods**: NGcGM3 expression and cell death were analyzed by PI incorporation and cytometry assays. Morphological and biochemical features of cell death were studied by microscopy, immunofluorescence, DNA extraction and western-blot. **Results**: 14F7 mAb-cytotoxicity was induced in murine tumor cell lines, but not in mouse normal cells (B and CD4+) that expressed the antigen. F(ab)´2 but not Fab fragments retained the cytotoxic capacity of the whole 14F7 mAb. Impairment of ganglioside synthesis in tumor cells abrogated the 14F7 mAb cytotoxic effect; however exogenous reincorporation of the ganglioside did not restore tumor cell sensitivity to 14F7 mAb-induced cytotoxicity. Interestingly, this complement-independent cell death mechanism did not resemble apoptosis, since no DNA fragmentation, Fas mediation or caspase activation was observed. However NGcGM3 ganglioside-mediated 14F7 mAb-induced cell death was accompanied by cellular swelling, membrane pore formation and cytoskeleton activation, suggesting an oncosis-like phenomenon. **Conclusions**: This novel mechanism of cell death let us to support further therapeutic approaches using NGcGM3 as a molecular target for antibody-based cancer immunotherapy.

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PIP02- ALTERATIONS OF TH1, TH2 AND TH3 POLARIZATION IN MAJOR DEPRESSION: EFFECT OF SERTRALINE THERAPY

Oktenli C[1], **Sutcigil L**[2], **Musabak U**[3], **Cansever A**[2], **Uzun O**[2], **Sanisoglu SY**[4], **Bozkurt A**[2], **Yesilova Z**[5], **Ozmenler N**[2], **Ozsahin A**[2], **Sengul A**[3].

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Introduction: The specific associations between antidepressant treatment and alterations in the levels of cytokines remain to be elucidated. In this study, we aimed to explore the role of IL-2, IL-4, IL-12, TNF-α, TGF-β1 and MCP-1 in major depression and to investigate the effects of sertraline, a selective serotonin re-uptake inhibitor (SSRI) antidepressant, therapy. **Materials & Methods:** Cytokine and chemokine levels were measured at the time of admission and 8 weeks after sertraline treatment (50-100 mg/days). All immunological parameters were determined by Enzyme Immunoassay method. The severity of the depression was quantified by the Hamilton Depression Rating Scale (HDRS) on pre- and post-treatment with sertraline. **Results:** Our results suggest that the mean levels of HDRS ($P < 0.001$), IL-2 ($P < 0.001$), IL-12 ($P < 0.001$), TNF-α ($P < 0.001$) and MCP-1 ($P < 0.001$) were significantly higher in patients with major depression than in controls. As compared with the controls, the mean levels of IL-4 ($P < 0.001$) and TGF-β1 ($P < 0.001$) were significantly lower in patient group. The mean levels of HDRS ($P < 0.001$) and IL-12 ($P < 0.001$) decreased significantly after the sertraline treatment, whereas IL-4 ($P = 0.001$) and TGF-β1 ($P = 0.010$) increased significantly. **Conclusion:** In conclusion, our results indicate that Th1, Th2, and Th3 are altered in the depressed patients and are some of them corrected by sertraline treatment. These results support the concept that depressive disorders have been associated with changes of various aspects of the immune response, both immunoactivation and immunosuppression.

PIP03- INDUCTION OF TRANSPLANTATION TOLERANCE BY ALLOGENEIC DONOR-DERIVED $CD4^+CD25^+FOXP3^+$ REGULATORY T CELLS

Velásquez-Lopera M[#‡§], **Eaton V**[#†], **Lerret N**[#†], **Correa L**[§], **DeCresce R**[#], **García LF**[‡], **Jaramillo A**[#].

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Syngeneic $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs) are involved in transplantation tolerance. However, it is not clear whether allogeneic Tregs are able to regulate T cell alloreactivity. To establish whether donor-derived allogeneic Tregs are able to regulate alloreactivity, C57BL/6 $CD4^+CD25^-$ and $CD8^+$ T cells were cultured with BALB/c dendritic cells (DCs) in the presence of C57BL/6 (syngeneic), BALB/c (allogeneic), or C3H (third-party) Tregs. Alloreactivity was evaluated by IFN ELISPOT. Syngeneic and allogeneic, but not third-party Tregs, were able to significantly inhibit $CD4^+$ and $CD8^+$ T cell alloreactivity. Similar results were obtained in the opposite combination, BALB/c T cells and C57BL/6 DCs, indicating that regulation is not restricted to a specific genetic background. The effect of allogeneic Tregs was IL-10- and TGF-dependent and reversed by exogenous IL-2. *In vivo*, syngeneic and allogeneic donor-derived, but not third-party Tregs, induced donor-specific tolerance and enhanced C57BL/6 skin allograft survival in $CD4^+CD25^-$ T cell-reconstituted BALB/c *nu/nu* recipients. Additionally, human $CD4^+CD25^-$ T cells were cultured with allogeneic PBMCs and Tregs. Autologous, allogeneic, and semi-allogeneic third-party, but not fully-allogeneic third-party Tregs, inhibited T cell

alloreactivity. These results indicate that both murine and human allogeneic donor-derived Tregs inhibit T cell alloreactivity and suggest their potential use in the induction of transplantation tolerance.

PIP04- T CELLS ARE CRUCIAL FOR THE ANTI-METASTATIC EFFECT OF ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR ANTIBODIES

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Experimental evidences supporting the epidermal growth factor receptor (EGFR) as an important molecule for tumor metastasis had been accumulated. Currently, anti-EGFR monoclonal antibodies (mAbs) constitute a promising approach for the treatment of patients with metastatic tumors. However, the mechanisms associated with the potent anti-metastatic effect of these mAbs have not been completely elucidated due to the lack of appropriate syngeneic preclinical models. We have investigated the effects of 7A7, an antibody specific to murine EGFR, on the metastatic properties of D122 murine lung carcinoma. 7A7 mAb significantly impaired metastatic spread of D122 cells in C57BL/6 mice by direct anti-proliferative and pro-apoptotic effects on tumor metastasis. 7A7 mAb capacity to inhibit EGFR activation on D122 cells could contribute to its anti-metastatic effect. In addition, 7A7 mAb was able to induce *in vitro* antibody-dependent cell-mediated cytotoxicity on D122 cells. Interestingly, 7A7 mAb treatment increased the number of natural killer cells, T lymphocytes and dendritic cells infiltrating the metastatic sites. More strikingly, depletion of $CD8^+$ and $CD4^+$ T cells *in vivo* completely abrogated the 7A7 mAb anti-metastatic activity whereas function of natural killer cells was irrelevant. This study supports an *in vivo* role for T cell response in the mechanism of action of anti-EGFR mAbs, suggesting the induction of an adjuvant effect.

PIP05- SPECIFIC IMMUNE RESPONSE INDUCED BY IMMUNIZATION WITH THE AUTOLOGOUS EPIDERMAL GROWTH FACTOR RECEPTOR-EXTRACELLULAR DOMAIN

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The Epidermal Growth Factor Receptor (EGFR) plays a central role in regulating neoplastic processes. The EGFR overexpression in many human epithelial tumors has been correlated with disease progression and bad prognosis. Passive EGFR target directed immunotherapy but not active specific approaches have already been introduced in medical Oncology practice. Then we wonder if mice immunization with the murine EGFR-extracellular domain (ECD-mEGFR) in adjuvants can circumvent the tolerance to self EGFR inducing an immune response with biological effects over EGFR+ tumor cells. The present study demonstrated that despite mEGFR expression in thymus, strong DTH response was induced by the immunization with the ECD-mEGFR in Complete Freund Adjuvant (CFA), similar to KLH, a strange protein for mice. Besides, the immunization with the ECD-mEGFR in CFA or Very Small Sized Proteoliposomes from *Neisseria meningitidis* (VSSP) induced highly

specific IgG titers, with IgG2a and IgG2b isotypes. In addition, mice were immunized with the ECD of human EGFR (ECD-Her1) to compare the self-recognition induced by heterologous and autologous immunization. Immunization with ECD-Her1 in VSSP induced elevated specific IgG titers, while cross-reaction with the ECD-mEGFR was undetected. Sera from mice immunized with ECD-mEGFR/VSSP or ECD-Her1/VSSP not only recognized by FACS EGFR+ murine or human tumor cell lines, respectively, but also inhibits the *in vitro* growth of those cells. The anti-metastatic effect of the autologous vaccine was dependent of CD8+ T cells, without any reproductive side effects. Our results suggested that ECD-Her1/VSSP may be used as a vaccine for patients with EGFR+ tumors.

PIP06- PHARMACOLOGIC EVALUATION OF A NOVEL PROAPOPTOTIC PEPTIDE THAT IMPAIRS THE PROTEIN KINASE (CK2) PHOSPHORYLATION IN TUMOR ANIMAL MODELS

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Introduction: We have previously identified a novel proapoptotic peptide that impairs the CK2-mediated phosphorylation and exhibits antineoplastic properties in vitro and in vivo[1]. Here, we explored the pharmacodynamic effect of this peptide when administered systemically in tumor animal models. Also, data from biodistribution, immunogenicity and toxicology of P15-Tat (also termed CIGB-300) is discussed. **Materials & Methods:** P15-Tat was injected in a consecutive 5-day schedule through either intraperitoneal or intravenous route in three relevant cancer models (syngeneic murine model TC-1/C57BL6, and human xenografts models Hela/Nu mice and H-125/Nu mice). Pharmacodinamic effect was registered considering tumor mass volume and survival time. For biodistribution studies, ^{99m}Tc-labeled P15-Tat accumulation on tumors was registered at 0.5, 4 and 24 hrs after injection of 10 mg/Kg dose. In situ DNA fragmentation on tumor was detected using a DeadEnd Flourometric TUNEL System and used as pharmacodinamic marker. Immunogenicity of P15-Tat was evaluated by ELISA. **Results:** Significant delay of tumor growth was observed at 2 mg/kg, 10 mg/kg or 40 mg/kg after P15-Tat administration in both syngeneic and xenografts models by systemic administration. Accordingly, ^{99m}Tc-labeled P15-Tat peptide was certainly accumulated on the tumors (1-3%) after administration by both routes and it induced apoptosis in the tumor as evidenced by the in situ DNA fragmentation assay. **Conclusions:** This report becomes the first describing the pharmacodynamic evaluation of a peptide that targets the acidic phosphorylation domain for CK2 substrates by the systemic administration. Also, our data reinforces the perspectives of P15-Tat for the cancer targeted therapy of anatomically hard-to-access tumors.

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PIP07- ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR ANTIBODIES DECREASE TUMOR GROWTH PROMOTED BY RADIATION THERAPY

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Introduction: Because EGF receptor has been reported to be a radiation response modulator, HER inhibitors are regarded to act as potential radiosensitizers. Our study assessed the potential role of two anti-EGFR monoclonal antibodies (mAb), as radiosensitizers in a murine glioma model when combined with radiotherapy *in vivo*. **Material and methods:** U87MG is a human glioblastoma cell line. Human/mouse chimeric anti-EGFR mAb Cetuximab (ImClone Systems, NY). Humanized anti-EGFR mAb Nimotuzumab (CIM, Cuba[1]). Immunohistochemistry, Proliferation Ki-67, Angiogenesis, Apoptosis, and Immunoblotting. Animal experiments were conducted in female NMRI athymic mice (Charles River, Germany). **Results:** The antibodies were safe and well tolerated administered alone or combined to radiotherapy. Co-administration of Nimotuzumab or Cetuximab with radiotherapy increased the radiosensitivity of U87MG resulting in a significant delay of subcutaneous (*s.c.*) tumor growth. Furthermore, add the antibodies to the radiation decreased brain tumor sizes and inhibited by 60 to 66% the increased tumor cell invasion provoked by radiotherapy, while promoted tumor cell apoptosis. Whereas Nimotuzumab reduced the tumor blood vessel formation in *s.c.* tumors, Cetuximab had not effect. Moreover, Nimotuzumab combined with radiotherapy reduced the tumor cell proliferation and inhibited EGFR and ERK1/2 signalling. In contrast, Cetuximab induced a more marked inhibition in the phosphorylated EGFR and ERK1/2 protein expression alone or combined to radiotherapy. **Conclusions:** Both antibodies increased radiosensitivity in the human U87MG glioma tumor xenografted subcutaneously and orthotopically in NMRI nude mice by different mechanisms. Furthermore, our results suggest that anti-EGFR agents-based therapies might prevent the tumor cell invasion induced by radiation alone in the treatment of glioma tumors. **References: 1.** Mateo, C. et al. (1997) Immunotechnology 3: 71-81.

PIP08- ASSESSMENT OF CANDIDATE IMMUNOPOTENTIATOR CM-95 SOLUTION UNDER MAGNETIC TREATMENT OVER PARAMETER BLOODY-RED AND TISSUE OF BALB/C MOUSE

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Introduction. The use of technology with magnetic field as waterish system under magnetic treatment constitute applications of biomagnetic for medicine, among them, the CM-95 solution under magnetic treatment to show forth stimulant effect over the humoral and cellular response in experimental biomodel, so that, have been allowed its use as to immunologic adjuvants for obtenir biology product, and its assessment as a candidate to immunopotentiator pharmaco. **Materials and Methods**. For complete the preclinics studios the CM-95 saline solution, treatable with magnetism in the interval of 0,01-0,16 T, was inoculated 0.2 ml volume in balb/c mouse by intraperitoneal way. The same solution without treatment and animals without inoculate as control. In the

analyst automatic Hitachi 902, were analyzed totals proteins, glucose, creatinine, transaminase ASAT and ALAT, calcium ionic, cholesterol, and total quantification and diffential of leukocytes. The macrophages were aisled by peritoneal exudates and quantificate in neubawer camera. **Result** was evident, the CM-95 solution treatable magnetically permits changes in the bloody-red leukocytes, Ej elevate the lymphocytes and macrophages peritoneals in relations controls, and so the biochemistry parameter analyzed, but all among normal valoures for the species. **Conclusions**. The CM-95 solution treatable magnetically induce pharmacology effect over bloody-red and tissue parameter the importance for cellular metabolism and the response immune.

PIP09- REDUCTION OF ACETAMINOPHEN-INDUCED HEPATOTOXICITY BY PRE-TREATMENT WITH FREUND´S ADJUVANTS IN MICE

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Acetaminophen is frequently used for the relief of pain and fever in infants and peoples of all age groups after vaccination and infectious diseases. This study was designed to examine the effect of Freund´s adjuvant-induced inflammation, on the hepatotoxicity for overdoses of acetaminophen in female Balb/c. **Methods:** Five animals were injected subcutaneously with Freund Complete Adjuvant (FCA) and boosted with Freund Incomplete Adjuvant (FIA) at 14th day, and treated with 360 mg/kg of acetaminophen orally during 14th, 15th and 16th days. The histopathological responses were evaluated in the livers and biochemically serum alanine transaminase (ALT), aspartic acid aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) levels were also determinate **RESULTS:** FCA/FIA injection led to the development of an acute inflammatory response in the site of inoculation and was noted a significant increment of ALT and AST in the acetaminophen-treated group associated with hepatic centrilobular necrosis signs induced by the overdose. In contrast in the group with co-administration of adjuvants the histological damages were less evident and the level of both enzymes were lower than that positive control group, evidencing a hepatoprotective effect provoked by the immunostimulation. On the other hand the level of AP and LDH were significant higher in the group with co-administration of acetaminophen plus FCA in comparison with the other groups. In **conclusion** our results indicated that inflammation induced by Freund´s adjuvant was able to reduce hepatotoxicity for acetaminophen overdoses in the experimental conditions evaluated

PIP10- WOUND HEALING IN PRECLINICAL PHARMACOLOGY. INFLUENCE OF THE EXPERIMENTAL DESIGN OVER RAT IMMUNE SYSTEM MAIN ORGANS MORPHOLOGY

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In this communication we show the histological results of the skin, thymus and spleen in an experimental design of 4 total thickness wounds on the rat dorsal region. The group without wounds was sacrificed at 7 days while the Placebo and all original treated groups with a X product were subdivided in 3 animals, sacrificed at 4 days and 3 at 7 days (n=6). The samples of the wounded skin were obtained from the original groups, stained with Haematoxilin-eosin and Masson`s Trichromic technique while the samples of thymus and spleen were taken from 4 groups (negative control, placebo and 2 treated, selected at random).The body weight was recorded in every point of sacrifice. It could not be found a complete healing in any animal of the whole groups of the experiment by means of a semiquantitative scale (Berlanga et al. 1997, modified by Merino, 2000) with a

reduction of the body weight at 4 days ($p<0.05$). The histology of the thymus in the selected groups revealed a decrease of the cortex and the morphometry showed a significative difference in comparison with the control group ($p<0.05$). In the spleen was demonstrated a decrease of the PALS as a t dependent region and an absence of the Mantle zone at the lymphoid follicles (b dependent). We conclude that the number and size of the wounds is the cause of the increased pain during the inflammatory phase of the healing and there are stress factors that delay or inhibit the event of a normal healing and therefore we recommend not only to decrease the number of the wounds but to add the histological study of the thymus and spleen that can be useful as sentinels to guaranty the results of pharmacological study of the evaluated product.

PIP11- POTENTIAL BIOCHEMICAL SERUM MARKERS OF VASCULAR INJURY IN CHRONIC CYCLOSPORINE TREATED RATS

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Cyclosporine (CsA) is a potent immunosuppressant agent that has been extensively used in transplanted patients. Vascular disease is a major cause of morbidity and mortality in transplant recipients, but the influence of CsA on mechanisms underlying vascular injury are poorly understood. Here, we investigated, in non-transplanted rats, risk factors for vaso-occlusive disorders, changes in vascular morphology and inflammatory parameters induced by chronic CsA administration. We observed that CsA treatment by daily gastric gavage during 8 weeks caused inflammatory vascular disturbances that were corroborated by changes in serum biochemical markers (homocysteine, ectonucleotidases and uric acid) as well as morphological alterations in the aorta and carotid artery. Thus, we suggest that these serum biochemical parameters appear to be promising markers of vascular disturbance in the monitoring of CsA therapy.

PIP12- EVALUATION OF THE *in vitro* VERO CELL ASSAY AS ALTERNATIVE TO THE *in vivo* TOXIN NEUTRALIZATION TEST FOR DIPHTHERIA VACCINES POTENCY

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The diphtheria toxin causes a dermonecrotic effect on rabbit and guinea pig skin and it has cytotoxic activity on Vero cell. Both properties have been used for titration of diphtheria antitoxin in immunized guinea pig serum by in vivo and in vitro neutralization methods. The in vivo toxin neutralization is used as antibody test for control testing of diphtheria potency in vaccines. This test requires at least two guinea pigs for estimating diphtheria antitoxin titre. The Vero cell assay based on the cytopathic effect of the residual diphtheria toxin was evaluated as alternative of the in vivo assay. A linear relationship was observed between toxin dose levels and concentrations of antitoxin (1, 0.1, 0.001, 0.0001 UI/mL). At 0.001 and 0.0001 UI/mL concentration antitoxin the dose levels of toxin were 2 times less than the expected concentration. These results indicate that the equine antitoxin standard didn't have enough avidity to completely neutralize toxin at low reactant concentrations. Vero cell method yielded coefficients of variations less than 20 % from 3 independent experiments, showing a high reproducibility. Parallel titration of serum samples from guinea pig immunized with 23 lots of DT and DTP vaccines showed a significant correlation (Spearman`s correlation coefficient of 0.827) between in vivo and in

vitro tests. Hence, we can use the Vero cell test as an alternative for determining Diphtheria potency in vaccines.

PIP13- EVALUATION OF ANTIPROLIFERATIVE EFFECT OF THE IFNα/C-PHYCOCIANIN COMBINATION IN HEP-2 TUMOR CELLS

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Introduction: Conventional cancer therapies, including surgery, chemotherapy, and radiotherapy, as single modalities have a limited but important role in the overall treatment of most solid tumors. Thus, the strategies of cancer treatment using combined therapies or combined agents with distinct molecular mechanisms are considered more promising for higher efficacy, resulting in better survival. C-phycocyanin (C-phyco) is a major biliprotein of *Spirulina platensis,* blue-green algae possessing various biological and pharmacological properties. C-phyco acted as an antioxidant, citoprotective, immunomodulatory and antitumoral agent. Interferons (IFN) represent a large class of agents with antiviral and antitumoral activity. The action of IFN derives from a direct effect on the cell cycle, an inhibition of growth factors production and an anti-angiogenic effect. We evaluated the effect of both agents on the basis of its antitumoral properties up to that time demonstrated independently. **Material and methods:** Hep-2, a human laryngeal tumor cells were grown in DMEM supplemented with 10% fetal calf serum; the growth of Hep-2 cells treated with C-phyco, IFNα and IFNα/C-phyco combination was measured by MTT assay in pre and co-treatment scheme. **Results:** Treatment of tumor cells with each of the agents alone decreased cell proliferation. In general, C-phycocyanin when used in combination with IFNα produced synergistic inhibitory effect on proliferation of Hep-2 cells. **Conclusions:** The results provide a further basis for the use of combinations of C-phyco with IFNα in the treatment of certain types of solid tumors.

PIP14- EFFECT OF *Mangifera indica* L EXTRACT (VIMANG) ON HUMAN LUNG (H460) AND COLON (HT-29) TUMOR CELLS PROLIFERATION

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Vimang is a mangiferin-enriched extract obtained from the stem bark of *Mangifera indica* L. (mango), which shows potent *in vitro* and *in vivo* anti-inflammatory and antioxidant activities. It was recently shown that mangiferin-mediated down-regulation of NF-κB potentiates chemotherapeutic agents-mediated U-937 human histiocytic lymphoma cells death (Sarkar et al., 2004), suggesting a role in combination therapy for cancer. Considering that mangiferin account for around 20% of Vimang, we decided to test the effect of the extract alone or in combination with cisplatin on human colon and lung cancer cells. The results show a dose-dependent proliferative effect of Vimang on H460 and HT-29 cells at doses from 1 to 5 µg/ml mangiferin equivalent. Higher doses of the extract (10-50 µg/ml) induced cancer cells death, mainly by apoptosis. The results show no synergisms between the extract and 1µM cisplatin. We also show for the first time that Vimang increased the levels of BDNF at low doses but diminished it at high ones in H460 (lung cancer cells). At 50 µg/ml mangiferin equivalent the extract also reduced the levels of NGF in this cell type. We did not observed any effect of Vimang on neurotrophins levels in HT-29 cells (colon cancer cells). The inhibitor of Trk (neurotrophins receptors)

phosphorylation K255a did not modify the extract effect on cancer cells proliferation/viability. Vimang also increased the levels of TNF-α dose-dependently in both cells line, an effect that could explain the diminution in the neurotrophins levels and the cytotoxic effect associated to higher doses of the extract. **Reference:** Sarkar A, et al. J Biol Chem 279**:**33768-33781; 2004.

PIP15- *In vitro* STUDY OF IMMUNOMODULATORY ACTIVITY OF AQUEOUS INFUSION OF *Bidens pilosa*

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Introduction. *Bidens pilosa* is an annual plant from tropical America with anti-inflammatory properties in hepatitis, laryngitis, headache and digestive disorders, among others. Its wide pharmacological applications can be attributed to its chemical composition, with inhibitory effects on pathogenic microorganisms, which show strong antioxidant capacities. The objetive of this woks was study of the immunomodulatory activity of an infusion of this plant. **Material and methods**.The effect of the extracts on the production of tumor necrosis factor α (TNF α) and interleukin-1β (IL-1 β) by whole blood cells stimulated with lipopolysaccharide (LPS) from *Escherichia coli* and *Salmonella abortus equi*. Hundred microlitre of endotoxin solution (10 µg/ml) was added to 200µL of human blood, 100 µL of the infusion and 800 µL of sterile saline solution in sterile polypropylene tubes. The assay mixture was incubated at 37 ◦C for 18 h in a thermoblock. Cell-free supernatants obtained by centrifugation at 3000×*g* for 1 min were stored at −80 ◦C until cytokine measurement.The viability of the cells after exposure to the extracts was evaluated by the trypan blue exclusion test **Result**. The aqueous infusion of *Bidens pilosa* showed an increase in the cytokine production after stimulation with LPS from *E.coli*. This increase in the amount of cytokine would be due to the antimicrobial activity of the infusion. The increase in the TNF production induced by *Bidens pilosa* was about 1.86 times in the presence of LPS and 2.56 times without LPS. TNFα is one of the central regulatory cytokines in the induction of macrophage antimicrobial activities.**Conclusion.** The Bidens pilosa infunsion was able to increased cytokine production in whole blood stimulated or not by lipopolysaccharides (LPSs).

PIP16- *In vitro* LEISHMANICIDAL ACTIVITY OF ELEVEN ARTEMISIA SPECIES OF IRAN

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Introduction: Leishmania major is responsible for the cutuneous leishmaniasis. Many people are infected or living at risk of infection with leishmania parasites. The drugs for the treatment of leishmaniasis have unpleasant side-effects or are not effective. Thus, the development of new and effective leishmanicidal agents is urgently needed. Previously, leishmanicidal activity of some *Artemisia* spp. was reported but other species were not determined. The objective of this study was to investigate the leishmanicidal activity of 11 *Artemisia* spp. from Khorasan Province. **Materials and Methods:** Eleven species of *Artemisia* were collected from Khorasan Provinces and their ethanol, ethylacetate, dichloromethan and hexan extracts were prepared. Leishmania major promastigotes were cultured in vitro. Leishmanicidal effects of these extracts were evaluated by MTT assay and

reported as 50% inhibition concentration (IC_{50}). **Results:** All extracts were inhibited proliferation of promastigotes in a dose-dependent manner. Ethanol extracts had the strongest effect and hexan extracts (except for *A.fragrans*) had the weakest effect. Ethanol extracts of *A.kulbadica* (IC_{50}:0.025), *A. ciniformis* (IC_{50}:0.025) and *A. santolina* (IC_{50}:0.080) had the most leishmanicidal activity. All ethylacetate extracts (except for A.fragrans and A.turanica) were stronger than dichloromethan extracts. **Conclusions:** Our results demonstrated that *Artemisia* spp. from Khorasan Province could be good candidates for the investigation of leishmanicidal activity *in vivo*. So, isolation of effective compounds and elucidation of their structures will be essential.

PIP17- THE CYTOTOXIC EFFECTS OF SOME FRACTIONS ISOLATED FROM *Pleurotus florida* BODY EXTRACT ON CANCER CELL LINES

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Introduction: Natural plant compounds can have preventive effect against cancers. Mushrooms are nutritionally functional food and the most significant medicinal effect of them is their anti-tumor property. Today, different medical approaches are used for the treatment of cancers, but in most cases they are not effective or have unpleasant side-effects. This forced scientists to study for more effective drugs with less toxicity. This study was evaluated the cell cytotoxicity effect of some fractions isolated from *Pleurotus* florida on cancer cell lines. **Materials and Methods:** R5, F5, R10, R30 and R100 fractions isolated from *Pleurotus* florida body extract. The growth inhibitory activity of these fractions was determined for cancer cell lines including gastric adenocarcinoma cell line (AGS), renal adenocarcinoma cell line (ACHN), cervical cancer cell line (Hela), colon adenocarcinoma cell line (HT-29) and fibroblast cell line (L929) using colorimetric MTT assay. **Results:** The results showed that the isolated fractions tested in this study showed significant inhibitory activity for cancer cell lines in a dose-dependent manner. Some of the fractions such as R100 and R30 exhibited the most inhibitory activity against HT-29. Among the cell lines tested, HT-29 was very sensitive to these fractions. **Conclusions:** In this study a series of fractions isolated from *Pleurotus florida* extract had cytotoxicity effects on cancer cell lines. All fractions had the most cytotoxicity effect on human colon cancer cell line (HT-29). Fraction R100 had the most cytotoxicity on it. Further studies are needed to elucidate the mechanisms by which R100 fraction act.

PIP18- STUDY THE CYTOTOXICITIC AND PRO-APOPTOTIC EFFECTS OF *Pleurotus florida* BODY EXTRACT ON CANCER CELL LINES

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Introduction: Natural plant compounds can have preventive effect against cancers. Mushrooms are nutritionally functional food and the most significant medicinal effect of them is their anti-tumor property. Different medical approaches are used for the treatment of cancers, but in most cases they are not effective or have unpleasant side-effects. This study was evaluated the cytotoxicity effect of water extract from *Pleurotus* florida body on cytotoxicity of some cancer cell lines. Also, we evaluated the pattern of cellular death in sensitive cell line.

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Materials and Methods: Cancer cell lines were provided by Natural Cell Bank of Iran and incubated in culture medium. Water extract was prepared from *Pleurotus* florida body. The growth inhibitory activity of this extract was determined for gastric adenocarcinoma cell line (AGS), renal adenocarcinoma cell line (ACHN), cervical cancer cell line (Hela), breast adenocarcinoma cell line (MCF-7), Adrenal fibroblast-pheochromocytoma (PC-12), hepatocyte carcinoma (HepG) and fibroblast cell line (L929) using MTT assay. Apoptotic cells were determined using AnnexinV-FITC and propidium iodide staining by flowcytometry. **Results:** The results showed that the water extract showed significant inhibitory activity for cancer cell lines in a dose-dependent manner. It exhibited the most cytotoxicity effect against AGS. This toxicity was induced by apoptotic and non-apoptotic cell death in AGS cell line. **Conclusions:** Edible mushroom, *Pleurotus* florida had cytotoxicity effect on cancer cell lines especially AGS through apoptotic and non-apoptotic cell death. Further studies are needed to elucidate the mechanisms by which this extract acts.

PIP19- IMMUNE RESPONSE IN MICE BALB/C TO RECOMBINANT *Streptomyces lividans* SECRETING *Mycobacterium tuberculosis* APA AND THE WILD TYPE STRAIN AS A LIVE VECTOR FOR VACCINE PREPARATIONS AGAINST TB

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According to the World Health Organization it is needed more than one vaccine: one to be delivered to infant, one to previously infected people and other with therapeutic function needed in people who has developed the active disease. As a result of this necessity, the enormous effort of the scientific community in the last 10 years has generated a great number of vaccine candidates against TB to be tested in different laboratory experiments, experimental animal models and clinical trials in human populations. Recently a new microbial live vector has become interesting for us as a reasonable strategy for developed TB vaccines: *Streptomyces lividans*. This bacterium belongs to one of the major branch of the Gram-positive bacteria: the high G-C organism referred to as the actinomycetes. *Mycobacterium tuberculosis* belongs to a suprageneric group inside the actinomycetes genera, the Mycobacteriaceae family, which is phylogenetically closed from the Streptomycetaceae family. This work is related with the studies of the patent (International Application No. PCT/CU2005/000002): "Vaccine compositions which are obtained from *Streptomyces." Streptomyces* strains are well-known as not pathogenic bacteria due to be a source of antibiotics for many years; and are characterized by their capacity to produce secreted proteins. We have previously obtained the expression in *Streptomyces lividans* of the *M. tuberculosis* Rv1860 sequence corresponding with the 45/47 kDa protein, also known as alanine-proline rich antigen (APA), an immunodominant antigen which is secreted into the culture medium of S. lividans during natural infection and it is also produced by BCG. We have seen that the APA protein arise specific antibodies levels in almost 50 percent of a Cuban population patients. There is the hypothesis that appropriate immunization of a naive host with extracellular proteins would expand a population of lymphocytes able to recognize an infected host cell at some subsequent time when the host is infected with the pathogen. In this way we have studied the immunogenicity of recombinant *S. lividans* secreting APA protein and the wild-type strain compared with BCG in mice Balb/C in order to determinate if these live vaccine vectors are capable of inducing potent humoral and cellular immune responses that could be evaluated in futures challenged experiments with *M. tuberculosis*. Both strains were sufficiently immunogenic for letting high levels of antibodies against the proteins of their culture supernatant in the serum samples of mice immunized with them as mean of ELISA and Western blot assays, and both strains have the same cross-reactivity pattern of antibodies against *M. tuberculosis* as the vaccine strain BCG. The immunization with the recombinant strain let also title of antibodies against the heterologous antigen APA, suggesting the *in vivo* expression of the protein. Leukocytes isolated from the spleen of the

immunized animals were *in vitro* restimulated with secreted proteins of the recombinant and wild-type strains assessing levels of secreted Ifn-γ of 1319.1 y 2103.9 pg/mL respectively, one of the major cytokines of the Th1 pattern of T-lymphocytes. This study demonstrated the high immunogenicity of these strains of *S. lividans* in mice, proofing to be an attractive way to design vaccine preparation against TB.

PIP20- THE USE OF STREPTOMYCES FOR IMMUNIZATION AGAINST MYCOBACTERIAL INFECTIONS

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Tuberculosis (TB) is the first cause of dead associated with infection diseases. Different strategies include the bioinformatic are currently being tested to identify and improved vaccines against TB. Comparative genome study between *Streptomyces coelicolor* and *Mycobacterium tuberculosis* suggested, that both descend from a common *Actinomycete*. In this work we explore the capacity of the immunization with *Streptomyces lividans* to produce a protective antibodies response against mycobacterial infection. Firstly we compared the theoretical proteomes of *M. tuberculosis* H37Rv and *M. bovis* with that one from *S. coelicolor*. Comparison showed much similarity at the level of individual protein sequences, and among these Open Ready Frames there are genes encoding for membrane and secreted proteins. Then we recognized the intraperitoneal route as the most immunogenic of *Streptomyces* administration through a whole cells ELISA of *S. lividans.* Immunogencity and specificity antibody response of different groups of mice inoculated with BCG and with *Streptomyces lividans*, were evaluated with whole cells ELISA and Western blot assay. Cross reactivity and specific antibodies were detected in sera. We evaluated the role of the immune response elicited by the inoculation with *Streptomyces* in an animal model of infection with BCG. The CFU counts from lung of this group were significantly lower in relation with the negative control group. With this results, finally we evaluated the role of the immune response elicited by the inoculation with *Streptomyces* in an animal model of infection with *M. tuberculosis*. We not detected significant differences between the group inmunizated with *S. lividans* and the negative control group.

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PIP21- SYNERGISTIC STIMULATION OF PROLIFERATION OF U138-MG GLIOBLASTOMA CELLS BY GASTRIN-RELEASING PEPTIDE IN COMBINATION WITH AGENTS THAT ENHANCE CAMP SIGNALING

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Increasing evidence indicates that gastrin-releasing peptide (GRP) acts as an autocrine growth factor for brain tumors. However, it remains unclear whether the cAMP/protein kinase A (PKA) signaling pathway plays a role in mediating the mitogenic effects of GRP. We show here that GRP acts synergistically with agents that stimulate the cAMP/PKA pathway to promote proliferation of human gliobastoma cells. Treatment with GRP combined with the adenylyl cyclase (AC) activator forskolin, the cAMP analog 8-Br-cAMP, or the phosphodiesterase type IV (PDE4) inhibitor rolipram increased proliferation of U138-MG cells in vitro measured by MTT assay. None of the compounds had an effect when given alone. GRP receptor (GRPR) mRNA and protein expression in U138-MG cells was detected by reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry. The results suggest that GRP and the GRPR interact with the cAMP/PKA signaling pathway in stimulating cancer cell proliferation.

PIP22- ROLE OF AMNIOTIC MEMBRANE AS BIOLOGICAL CURATIVE ON SURGICALLY DAMAGED LIVER, UNDER THE INFLUENCE OF VERAPAMIL, IN RATS

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It was studied the influence of homogenous amniotic membrane (HAM) as a biological curative in surgically induced liver damage, in rats under treatment with calcium blocker verapamil, by histological evaluation. **Material and Methods**. Ninety six male adult rats (*Wistar*), were divided in groups: 1)-LH group: control-rats with liver damage; 2)-LHHAM group: rats with liver damage plus HAM; 3)-V group: rats with liver damage plus verapamil; 4)-VHAM group: rats with liver damage, under verapamil treatment and HAM**.** The HAM was obtained from 24 pregnant females. Surgeries were performed under ketamin-xilazine anesthesia, and HAM dressed the injured area. At the 10th, 20th, 30th and 40th days after the surgery, the animals were sacrificed and excised liver tissue to histological analysis. **Results**. The HAM underwent metaplasia, that is, it was transformed in an amniohepatic tissue, evidencing biocompatibility. It was observed a significant increase of hepatocytes in the verapamil groups. Hepatocytes count was respectively at the 10th, 20th, 30th and 40th after the surgery: V group: 24.8±0.65; 23.05±0.8; 18.64±0.79; 15.59±0.48. VMAH group: 27.0±0.65; 24.88±1.11; 20.33±0.67; 17.22±0.81. LHHAM group: 20.33±0.67; 20.78±0.43; 15.59±0.48; 14.33±0.48. LH group: 18.93±0.57; 16.13±0.6; 14.92±0.91; 14.79±0.55. **Conclusions**. The verapamil, calcium blocking, stimulated the liver regeneration and presented

hepatoprotective property. The amniotic membrane showed inductive and angiogenic, mitogenic activities. (Supported by FAPESP: 08656/2003)

PIP23- FUNCTIONAL REGENERATION OF THE SURGICALLY DAMAGED LIVER AFTER TREATMENT WITH AMNIOTIC MEMBRANE AND VERAPAMIL, IN RATS

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The influence of homogenous amniotic membrane (HAM) and verapamil (V) on the functional regeneration of surgically damaged liver was evaluated by the determination of the enzymatic activity of aspartate and alanine aminotransferase (AST, ALT) and alkaline phosphatase (ALP) in liver tissue and plasma. **Material and Methods**. Ninety six male adult rats (Wistar), were divided in groups: 1)-LH group: control-rats with liver damage; 2)-rats with liver damage plus HAM; 3)-V group: rats with liver damage plus verapamil; 4)-VHAM group: rats with liver damage, under verapamil treatment and HAM**.** The HAM was obtained from 24 pregnant females. Surgeries were performed under ketamine-xilazine anesthesia and HAM dressed the injured area. At the 10th, 20th, 30th and 40th days after the surgery, the animals were sacrificed, collected blood and excised liver tissue to biochemical analysis. **Results.** The enzymatic activity of AST and ALT were significantly lower in the VHAM and V groups, indicating a protective action of the verapamil, and helping the liver regeneration process. The ALP activity was higher in verapamil treated rats, possibly as a result of higher tissue reparation as compared to not treated animals. **Conclusion**. Biochemical results indicate that verapamil stimulated the liver regeneration process. (Supported by FAPESP: 08656/2003)

PIP24- EVALUATION OF THE EXPERIMENTAL REDUCTION AND REMOVING OF THIOMERSAL OVER THE EFFICACY AND SAFETY OF VACCINES FOR HUMAN USE

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Thiomersal is an Organo – mercury preservative included in vaccines for human use. In Cuba, the most of vaccines that conforms the National Immunisation Program contain Thiomersal, including single – dose vaccine formulations like recombinant Hepatitis B and Meningococcal BC vaccines. Although there is no evidence of harm due to the level of exposure from vaccines, different manufacturers and regulatory bodies have promoted the reduction, substitution or elimination of Thiomersal. In fact, Thiomersal-free or reduced formulations are already available in the market. Nevertheless, issues concerning quality, safety and efficacy of vaccines could limit these options. The aim of this Paper is demonstrate that the elimination and or reduction of Thiomersal should not impact the quality of these vaccines. For that, we prepared some experimental Thiomersal-free and reduced vaccine samples by dialysis and evaluated the protein content (by Lowry method), in vivo/in vitro potency and effectiveness of the preservative against a Thiomersal-containing vaccine. There was no significant difference between Thiomersal-containing and non-containing samples regarding important quality parameters like protein content and potency for Meningococcal BC vaccines, but the Hepatitis B vaccines without Thiomersal yielded consistently higher potency values. At the same time, the formulations non-containing and reduced Thiomersal showed a satisfactory effectiveness of preservative, although not for all microorganisms.

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This approach provides useful and predictable information in order to produce large – scale single – dose batches with removed or reduced Thiomersal without affecting the quality, efficacy and safety of vaccines.

PIP25- CHARACTERIZATION OF REFERENCE MATERIALS FOR THE IMPLEMENTATION OF AN ELISA FOR DETERMINING ANTI-TETANUS ANTIBODIES IN GUINEA- PIG SERUM

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Tetanus toxoid vaccines have been able to reduce the mortality and morbidity caused by Clostridium Tetani worldwide, reaching high standards of quality, efficacy and security. During decades the vaccine potency for these vaccines has been based on its immunogenicity which final stage is a challenge test known by L+/10/50.This method possesses some limitations in terms of animal ethics and variability. That's why it is needed to developed alternatives for Tetanus Potency. One of the most relevant approaches is a serological evaluation with an ELISA for determining anti-Tetanus antibodies in guinea-pig serum. For the implementation of the Tetanus ELISA the generation of reference materials is required to assign values to the vaccine samples and reproduce the method to other laboratories. The present work aims to characterize working standards for the implementation of ELISA as an alternative for L+/10/50 method. For that we prepared and characterized a Tetanus Toxoid Standard for coating, three antisera used for calibration curve, negative and positive control purposes. Once characterised the standards, we standardized the ELISA by evaluating parameters like linearity and intra and inter precision. All parameters yielded satisfactory results according to reported for this kind of methods. Hence, we disposed of well-characterised standards and an ELISA for determining the biological activity of Tetanus Toxoid vaccines in a guinea-pig model.

PIP26- COMPARED STABILITY STUDY OF A TETANUS VACCINE WITH TRADITIONAL AND REDUCED ANTIGEN CONTENT. IMPACT OVER THE BIOLOGICAL ACTIVITY

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Tetanus Toxoid vaccines have been used for decades to prevent and reduce the incidence of the Clostridium Tetani infection. These vaccines must contain certain amount of antigen to guarantee a clinical protection in human beings. Nonetheless, there's a trend worldwide to reduce the Toxoid content in order to allow a balance between immunogenicity and safety. Anyway, this reduction should be performed with caution because any change in vaccine formulation must be supported by new stability studies addressed to evaluate if there are changes in the physico-chemical, microbiological and biological properties, as well as for establishing the storage conditions and shelf life of the new formulation. The aim of this Paper was to compare Vax-TET® batches formulated to the traditional antigen content (20 Lf/mL) and the reduced one (10 Lf/mL) through the evaluation of all quality parameters, mainly the biological activity determined by a challenge test known as in vivo neutralisation test. We performed two different stability studies: a shelf life study during 24 months at 2 - 8 ° C and an accelerated one for 6 months at 30 ° C for the new formulation. All parameters successfully passed their quality specifications in both studies and there was no significant difference between the formulations.

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There was no significant difference between both formulations regarding the neutralisation test results. We demonstrated that it can be used a Tetanus reduced formulation with no risk of instability of loss of immunogenicity during 2 years.

PIP27- CHARACTERIZATION OF WORKING REFERENCE STANDARDS FOR DETERMINING THE POTENCY OF TETANUS VACCINES ACCORDING TO WHO APPROACH

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There are two different approaches for the evaluation of Tetanus Vaccines Potency. WHO Technical Report Series for Tetanus Vaccines consider the lethal method as the most relevant Potency test. This method requires the use of well-characterized reference standards and reagents to assure reliable and consistent results. The aim of this work was to calibrate a Tetanus Toxin batch as reference reagent and an adsorbed DT combined vaccine batch as in-house reference standard. For the characterization of Tetanus toxin we make dilutions of the Toxin and inoculated them in groups of 10 mice, performing 12 independent tests. With these results we calculated the geometric mean and assigned a biological activity value in terms of LD50. The vaccine candidate was characterized by comparison with the 3rd International Standard of Tetanus Toxoid adsorbed (code: 98/552). Three vaccine dilutions were prepared and immunised in healthy animals. Twenty days later all mice were challenged with 50 LD50 of Tetanus Toxin. The individual test results were processed by Probit analysis. All the assays were valid, fulfilling the acceptance criteria (linearity and parallelism). Both standards were well-characterized (the vaccine standard in International Units per mL) and are available to standardize the WHO Potency method for combined vaccines containing Tetanus Toxoid.

PIP28- METHODOLOGY FOR THE FORECAST OF THE IMMUNOGENICITY / AUTOIMMUNITY OF EXOGENS PROTEINS

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Many biotech products to be administered to human with diagnostic or therapeutic purposes consist of proteins derived from microorganisms, plants and other animal species. As this is strange molecules, it is possible that the immune systems recognized as such and generate a response against them, which is the basis for undesirable reactions that can, on one hand, reduce the therapeutic use of the product in question, or on the other hand, mean a significant risk to the health or life of the patient. The present work shows a methodology for predicting the immunogenicity and the autoimmune nature of exogenous proteins based bioinformatics tools: obtaining the primary sequence of the substance by sequencing or from databases, and generation of peptides that selection can be submitted by molecules System Main Histocompatibility and finally, finding similarities with the human proteome. It describes the locations, advantages and benefits of major bioinformatics resources available for each stage: databases general and specific algorithms for predicting and finding similarities and homologies. We recommend the combination with algorithms for predicting conformational epitope.

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PIP29- REDICTION OF MOLECULAR MIMETISM BETWEEN RECOMBINANT STREPTOQUINASA AND HUMAN PROTEINS

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The mimetism is one of the strategies employed by micro-organisms to evade immune responses and successfully colonize the human host. However, cross-reactions can be generated instead of tolerance which has the effect of damaging tissues and cells themselves. The bacterial streptokinase is a product widely used in the early treatment of acute ischemic episodes of the coronary vessels in humans. In order to predict the mimetism between streptokinase and recombinant human protein proceeded to generate peptides of 9 and 15 residues in length from the sequence of the protein and using the database SYFPEITHI (http://syfpeithi. bmi-heidelberg.com /). Those were selected higher affinity for molecules System Main Histocompatibility frequently in Cuba, and were used in the search for similarity through algorithm FastA (http://www.ebi.ac.uk/fasta33/genomes.html) developed by the European Bioinformatics Institute, setting the basis Proteoma and the data set ***Homo sapiens***. It shows the human protein found with this methodology, including those of the probable effect tolerogénico, and we discuss the possible cross-reactions.

PIP30- REGULATORY IMMUNE RESPONSES INDUCED BY AN APL FROM HEAT-SHOCK PROTEIN 60

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Rheumatoid arthritis (RA) is a multisystem inflammatory disease that primarily affects synovial joints. Currently, there is no effective treatment for RA. From one of the main autoantigens involved in the pathogenesis of RA, several T cells epitopes were identified which were used to design altered peptide ligands (APL). We analyzed the pattern of T-cell responses induced by a panel of APLs in PBMC from RA patients. The $CIGB_{814}$ peptide was able to produce a substantial increase on IL-10 levels (an important regulatory cytokine) in almost all patients. Moreover, we study the effect of this peptide on clinical signs and histopathological damage in the AIA rat model. Rats were monitored for arthritis signs according to severity scores previously established in our lab. Our results shown that this peptide reduces significantly the inflammatory signs in arthritic joints. Also, in treated animals we do not observed any histopathological damage. Furthermore, we evaluated the effect of $CIGB_{814}$ peptide on mice immune system. CFSE labeled spleen and peripheral LN T_{reg} cells from Thy-1.1BALB/c mice were transferred to Thy-1.2BALB/c mice. Mice were inoculated with 50ug/mL of original epitope or $CIGB_{814}$ peptide on day 0 and 5. Spleen and peripheral LN cells from mice sacrificed on day 4 and 9 were preincubated with several mAbs to estimate different T lymphocytes subsets. Data revealed that $CIGB_{814}$ peptide expands T_{reg} cells while original epitope seems to recruit conventional effectors T cells. Altogether, data suggested that the $CIGB_{814}$ peptide might represent a novel treatment option to specifically modulate the immune system in RA.

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PIP31- PHARMACOKINETIC ALTERNATIVES APPLIED TO THE ESTABLISHMENT OF THE BIOLOGICAL OPTIMAL DOSING (BOD): EXPERIENCE WITH AND MONOCLONAL ANTIBODY ANTI EGF-R

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At present time, at the aim of find therapeutical applications to different biotechnological molecules, among them the monoclonal antibodies, still the Regulatory Agencies maintain a discussion on the different methodologies to apply. In this way, the Food and Drug Administration of U.S.A., has offer a guidance for the selection of the starting safe dosing for a Phase I Clinical Trial. In this document they present the inquietude about the use of pharmacokinetic alternatives (allometric approach, integrated PK/PD modeling, etc.), to obtain the Biological Optimal Dosing. In the present lecture, exposing pharmacokinetic alternatives in preclinical studies with nude mice, rat, rabbit and dog, combining with the pharmacodynamic evaluation of tumor growth, receptor expression, apoptosis, etc in an xenograft model; it presents the employed algorithms as an strategy for the development of the monoclonal humanized antibody hR3 (Nimotuzumab®, CIMAB S.A., Cuba), based in the results about the precedent molecule, the murine MAb ior egf/r3. It proposes an attack and maintenance dose, and comparing this prognosis to the clinical experience of the hR3, and a similar product of different manufacture, the chimeric monoclonal antibody C-225, (Cetusumab®), the results correlates as equivalents.

PIP32- SAFETY AND PHARMACOKINETIC EVALUATION OF ANTI EGF RECEPTOR HUMANIZED MONOCLONAL ANTIBODY NIMOTUZUMAB (HR3) IN LOCALLY ADVANCED BREAST CANCER TUMOURS IN THE NEOADYUVANT SETTING IN COMBINATION WITH CHEMOTHERAPY REGIMEN. PHASE I CLINICAL TRIALS PRELIMINARY RESULTS

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Background: Nimotuzumab (hR3) is an IgG1 humanized monoclonal antibody that recognized an epitope located in the extra cellular domain of the human EGFR. Clinical efficacy has been shown in adult with high grade gliomas and head and neck tumours. **Purpose**: The phase I study was designed to evaluate the safety and the pharmacokinetics (Pk) of the Nimotuzumab administered concomitantly with chemotherapy (AC) in patients with locally advanced breast cancer tumours in the neoadyuvant setting at 50, 100, 200 and 400 mg/dose, respectively (3 patients per cohort). The primary objective for PK analysis was the determination of the area under the serum concentration versus time curve (AUC) and the half live (t1/2). Pharmacokinetic parameters were estimated after the first and the last antibody infusion.
Results: The clinical trial is still ongoing, but the first 3 dosage levels (50, 100 and 200 mg per doses) were already evaluated. Treatment was well tolerated. The toxicity of chemotherapy did not increase after combining with nimotuzumab. No cardiotoxic events were found. All patients developed grade 1 or 2 skin rash after combining with chemotherapy. None of the cutaneus adverse events showed after administering nimotuzumab before chemotherapy. No HAMA response either IgG or IgM against murine residues of hR3 were detected.

Nimotuzumab showed a non linear, dose dependent pharmacokinetic with no differences between 1st and 10th dose. The 10th dose showed similar dose depending behaviour and t1/2 increased 5.4, 3.8, 2.6 times as compared to the first administration. The 200 mg dose showed the best clinical result since all tumours became resectable after treatment. **Conclusion**. Nimotuzumab administered concomitantly with chemotherapy was safe and well tolerated. No severe adverse reactions were detected and the antibody showed a low immunogenecity. Following treatment of more than 700 patients in Cuba, skin rash was observed for first time after using Nimotuzumab in combination with doxorubicin and cyclophosphamide. The rash was transient, not dose dependent and disappeared without medication**.**

PIP33- VALIDATION OF TWO IMMUNOASSAYS FOR HAMA AND PK STUDIES IN CLINICAL TRIALS WITH NIMOTUZUMAB THERAPY IN CANCER PATIENTS

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Introduction: Nimotuzumab is a humanized monoclonal antibody (mAb) specific to the EGFR with encouraging results in the therapy of several cancer of epithelial origin. Two immunoassays were developed and validated, a cellular ELISA (CELISA) was performed for PK parameters estimation while an ELISA system was develop to determine the human anti- murine antibodies (HAMA) response. This work was aimed at attaining validated methods to be used in clinical trials. **Materials and Methods: T**he validation parameters of both immunoassays (specificity, sensitivity, working range, linearity, parallelism, accuracy, precision, reproducibility, and the intra- and inter-assay coefficient of variations) were properly determined. **Results:** CELISA method: The accuracy test showed good consistency (mean value 98.23%). The sensitivity and the upper limit of the working range were 7.8 and 250 ng/mL, respectively. The linearity and parallelism were obtained within the working range. Good coefficients of variation (CV) for intra- and inter-assay variability were determined. Unspecific responses were not observed. HAMA ELISA method: All the CV were much lower than 20% when analyzing the anti γ- and μ-chain conjugates, in the following sample dilution ratios (i.e., 1:400 and 1:800). The index rates obtained for 1:800 sample dilutions were significant higher than those for the 1:400 dilutions, suggesting a higher sensitivity. All the patients developed a HAMA response. **Conclusions:** Both immunoenzymatic methods were validated and usefully for clinical application. Evaluation of its clinical usefulness is ongoing.

PIP34- OVER EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN ANAL CANAL CARCINOMA AFTER TREATMENT WITH RADIOTHERAPY

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Introduction. The incidence of the anal canal carcinoma has increased in the last years. Local recurrence presented after curative surgery continues being an important problem in its management even after adjuvant combined chemo radiotherapy treatment. Then, new molecular markers are needed to develop treatments addressed to specific targets like the EGFr pathway. In cancer the EGFr signalling system is deregulated and over expressed in a variety of epithelial tumours. However the reports on the expression of EGFr in anal carcinoma remain controversial. **Objetive.** To evaluate the expression of the EGFr in anal canal carcinoma before and after radiotherapy. **Materials and metods.** Archival paraffin embedded tissues from biopsy obtained

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before treatment in 26 patients, nine of which were also performed after radiotherapy, were taken. Five micrometer serial sections were obtained The slides were dewaxed and rehydrated as usually, 0,4% pepsine in 1 mol/L HCL was applied as antigen retrieval solution before being incubated with iorR3 Mab for 1h, followed by bionilated antirabit antibody and a ABC peroxidase complex kit. **Results.** Ten out of 26 tumors were epidermoid queratinizing carcinoma, 6/26 were no queratinizing carcinoma and 10/26 basaloid carcinoma. A moderate to intense recognition of iorR3 Mab was evidenced in the biopsies taken before treatment while a shift toward intense to very intense recognition, was observed in the biopsies taken after radiotherapy. **Conclusions.** A shift toward intense or very intense immunoreaction was evidenced after radiotherapy. **Recommendations.** The increased overexpression of the EGFr observed after radiotherapy made it possible to recommend this Mab to perform a clinical assay in combination with radiotherapy.

PIP35- PRIMING AND BOOSTING DETERMINANTS ON THE ANTIBODY RESPONSE TO AN EGF-BASED CANCER VACCINE

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Active induced immune response is an attractive approach for cancer therapy and control. Ideally, a cancer vaccine should induce a rapid and simultaneously a long-lasting response in vivo. Unquestionably, therapeutic formulations containing better carriers and adjuvants may contribute to this end. However, the optimization of priming and boosting strategies may also improve the immunopharmacological response. The human Epidermal Growth Factor (EGF) chemically conjugated to the carrier protein P64k from *Neisseria meningitides* in Montanide ISA51 adjuvant is under evaluation as a cancer vaccine, with encouraging results. It induces anti-EGF antibodies to reduce the growth rate of EGF-dependant tumors. We explored the influence of priming and boosting variables on the antibody response to the EGF-based vaccine in BALB/c mice. Priming with suboptimal vaccine doses delays the induction phase conducing to a rapid decline of the immunoresponse. However, fractioning of an apparently low dose but involving multiple anatomical sites at priming enhances the antibody response which persists on time. Additionally, a reduced time lag to boosting was associated to a lower antibody response. In general, manipulations improving the priming effect contribute to the long-term response. We conclude that several determinants influence the immunopharmacological response, which may contribute to improve cancer vaccine efficacy.

PIP36- IMMUNOCHEMOTHERAPY WITH ANTI-CD20 MONOCLONAL ANTIBODY RITUXIMAB IN THE TREATMENT OF RELAPSED INDOLENT LYMPHOMA

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Background: Rituximab is a chimeric murine/ human anti-CD20 monoclonal antibody capable of killing CD20+ lymphoma cells. Addition of Rituximab to chemotherapy has been shown to improve response rates and progresión free survival in patients with indolent lymphoma. We performed a review of our experience with Rituximab therapy for relapsed/refractory indolent lymphoma. **Methods:** We analyzed 32 patients with refractory/relapsing indolent CD20+ lymphoma treated with Rituximab alone or combined with chemotherapy between January 2001 and December 2005. . Eligibility requirements included at least one prior systemic therapy and measurable disease. Endpoints were progression-free survival, overall response, and toxicity.

Results: Median age was 49 years (range: 26-71) with prevalence of female sex. Histological subtypes were: follicular (n = 22), small lymphocytic lymphoma (n = 5), nodal marginal zone B-cell lymphoma (n = 2), and others (n = 3). The median number of prior treatments was 2 (range: 1 to 5). Overall response rates were 76% percent. The complete response was 22%, and the partial 54%. The 2-year progression-free and overall survivals were 55% and 69%, respectively. Grade 3-4 neutropenia and thrombocytopenia were the most frequent adverse events. **Conclusion:** The final analysis of this study confirms in patients with relapsed/refractory indolent lymphoma therapy with Rituximab is highly effective and provides a potential benefit with an acceptable toxicity.

PIP37- EXPERIENCE WITH ADVERSE EVENTS ASSOCIATED WITH ACM H-R3 (THERACIM®)

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The AcM h-R3 (TheraCIM®) is a humanized monoclonal antibody isotype IgG1, obtained by genetic engineering that recognizes epitope Her-1 of the extracellular dominion of the receptor of the human growth factor. This antibody has demonstrated to be effective for the treatment of neoplasic diseases and clinical studies have been performed with treatment for breast, lung, prostate and esophageal under good results. At the moment this product is only registered in head and neck tumors treatment. The present work has been performed to describe the adverse events associated with the administration of this product for the treatment of brain metastasis in patients with non small cell lung cancer (NSCLC) in advanced stages; it was a randomized, controlled clinical trial with two groups of treatment: The first one with TheraCIM® and other with conventional treatment. The baseline characteristics of the patients, as well as the frequency, intensity and relation of causality of the reported events were analyzed. The more frequent adverse events were: asthenia, anorexy, cephaleas and vertigos, being expected events because they were reported in clinical tests previous studies. Most of the adverse events were of slight intensity to moderate and they did not require the suspension of the treatment. In conclusion, the TheraCIM® is a drug that has demonstrated to be safe for the treatment of brain metastasis in patients with NSCLC in advanced stages.

PIP38- PRESENCE OF TITLES ANTI-INTERFERON ANTIBODIES A PRONOSIS FACTOR OF THE CLINICAL RESPONSE RECOMBINAT INTERFERON

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A low number of patients treated with IFN-alfa show neutralizing antibodies which seem to be associated to a reduction of clinical efficacy but it is totally false that it is a reason for suspending the treatment. It is proposed to determine the presence of anti-interferon neutralizing antibodies titles as a prognosis factor of the clinical response to interferon alpha-2b. There were reviewed 46 medical records with their corresponding data notebooks of all patients included in each one of the clinical trials which evaluate the application of this product

in the province of Villa Clara, Cuba. Sandwich immunoenzimatic (IFNalfa-2b/sample/peroxidasa-protein of specificity) high specificity assay of anti-IFN antibodies of the patients was taken, considering it positive when the title is above 20 neutralizing units/ml, determined every three months. Only a patient showed positive results but with satisfactory clinical response so we conclude that the presence of neutralizing antibodies should not be taken as an indicator of loss of therapeutic efficacy of interferon alpha-2b.

PIP39- ADJUVANT INTERFERON GAMMA IN PATIENTS WITH PULMONARY ATYPICAL MICOBACTERIOSIS: A RANDOMIZED CONTROLLED STUDY

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Background. A high resistance to antibiotics is described in atypical micobacteriosis, mainly *Mycobacterium avium* complex (MAC). **Material and methods.** To define the response of patients with lung disease to the treatment with Interferon (IFN) gamma as immunoadjuvant to the chemotherapy a randomized, double blind, placebo-controlled clinical trial was carried out. Eighteen patients received 1 x 10^6 IU of recombinant IFN gamma intramuscularly during 6 months, other 14 patients received the placebo. Sputum samples collection for direct smear observation and culture as well as routine clinical and thorax radiography assessments were evaluated during and after treatment. **Results and Discussion.** Groups were homogeneous at entry, MAC infection (> 94%) prevailed. A significant early improvement in respiratory symptoms and magnitude of lung lesions was obtained in the IFN group. Around one third of the patients died in the placebo group but only 11.1% in the IFN group. Although differences in bacteriology were not significant during treatment, in the placebo group some patients re-converted to positive during annual follow-up. A significant increment both in TGF-β and oxidative damage were observed in the placebo group. Two thirds of patients treated with IFN gamma were evaluated as complete responders, but only one third in the placebo group. Treatments were good tolerated, without severe adverse events. Fever and chills were more frequent in the IFN group. Three patients in the IFN group normalized their globular sedimentation rate values. These data suggest that IFN gamma is useful and well tolerated as adjunctive therapy in patients with pulmonary atypical micobacteriosis, predominantly MAC.

PIP40- PHARMACOKINETICS ALTERNATIVE FOR THE EVALUATION OF IFN A-2B PEYILATE MOLECULES

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Nowadays the therapeutic proteins are used in the treatment of so many pathologies with great results but limited due to some inconvenient they have, such as a fast elimination, highly immunologic among others. The peyilation consist on the linking of chemestry proteins with a multi (poli-(etilen-glicol) (GEP). Researchers have demonstrated through this method is possible to reduce the problems associated to therapeutic proteins. In this project is carried out comparison of two choices of the pharmacokinetic analysis with the purpose to evaluate son clues of IFN α-2b native and $GEP_{2,\ 40}$ IFN α-2b thorough the monocompartmental modelation and the non

compartmental analysis (NCA). This investigation was done to 16 New Zeeland rabbits; they were divided in 3 groups to whom were 150 µg/kg of IFN α-2b native and 150 µg/kg GEP $_{2, 40}$ IFN α-2b in their skins and 50 µg/kg and 50 µg/ kg IFN α-2b native in their vains respectively. The concentration of IFN was determinated trough an Elisa method. After that, we also determinate the absolute biodisponibility of the molecule of IFN α-2b native, it shown that less of the 50% of the administrated dosage in an extravascular way gets to the systemic circulation. As the monocompartmental modelation and as the non compartimental analysis have shown result of the peyilate molecules because it was better since the point of view of pharmacokinetics then the native. The comparison of to choice of the pharmacokinetics analysis have shown that there is not great differences with results, fundamentally in areas of physiology bases (Vd) and (Cl); they are very useful for further clinic researches.

PIP41- ADYUVANT THERAPY WITH RECOMBINANT INTERFERON-ALPHA 2B IN THE TREATMENT OF PATIENTS WITH HIGH RISK MELANOMA

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A randomized and open clinical trial was carried out at the ¨Camilo Cienfuegos¨ University Hospital of Sancti Spíritus with the objectives of assessing clinical efficacy, safety and monitoring the appearance of anti-interferon-alpha (anti-IFN-a) antibodies during a year of treatment. 16 patients were included with a diagnosis of high risk melanoma, in which recombinant interferon-alpha 2b (IFN-alpha 2b R) was used during one year. Two groups were made up: group 1 used a dose of 20 million units per square meter of body surface during 4 weeks, continuing with 6 million units twice per week during one year. Group 2 used 10 million units per square meter of body surface 3 times per week during the same time. The intramuscular route was used. Clinical efficacy was assessed taking into consideration the patient´s condition on discharge; adverse events were monitored and anti-IFN antibodies were determined before beginning treatment, at 3, 6 and 12 months. Clinical efficacy was higher in group 1 with 6 living patients and controlled on discharge (66,6%). A total of 153 adverse events were reported, of which 112 (73,2%) consisted of general symptoms, more frequent in group 1, in which 5 serious adverse events (6%) occurred that didn't lead to the definitive suspension of therapy. Anti-IFN antibodies appeared in two patients at 3 and 6 months of therapy. Five patients abandoned the study. It is concluded that IFN-alpha is an effective and safe product at the doses used in the treatment of this disease.

PIP42- USE OF RECOMBINANT HUMAN INTERFERON-ALPHA 2B IN HIGH-RISK MELANOMA PATIENTS

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Introduction: Melanoma is a malignant tumour considered the most frequently cause of death among the cutaneous illnesses. It is an immunogenic tumour and it has bad results under chemotherapy. For obtain a longer overall survival time it is associate surgery and medical treatment, in many cases the adjuvant treatment with interferon. The Interferons (IFNs) alpha are cytokines that have citotoxic, antiproliferative and immunomodulatory action on tumour cells through their action on the patient's immune system. **Material and**

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methods: This study shows the results of a phase III Cuban clinical trial, multicenter, open and randomized with two treatment groups. The objective was to evaluate the efficacy and safety of high-risk melanoma's treatment with human recombinant IFN alfa 2b (Heberon Alfa R, Heberbiotec), manufactured by the Genetic and Biotechnology Engineer Centre. **Results:** Fifty-eight patients were recruited from 11 clinical sites of the country, 25 of them were under treatment 1 and the other 33 under treatment 2. Both groups were treated with different doses during one year. As a result, an overall survival probability (mean) of 30.9 months in group 1 and 35.8 in group 2, with an internal confidence of 95%, was achieved. Regarding disease-free survival, it was 27 and 34 months respectively. The most frequent adverse events were flu-like symptoms: chills, fever, asthenia, anorexia and myalgia. **Conclusions:** The study product has demonstrated to be safe and have a positive effect in the increase of overall survival time and in the interval of disease free time, in high-risk melanoma patients.

PIP43- HASHIMOTO'S AUTOIMMUNE THYROIDITIS. RESPONSE TO IMMUNOSUPRESSOR THERAPY USING GLYCOCORTICOIDS

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The Hashimoto's Autoimmune Thyroiditis (HAT) is the most frequent specific – organ autoimmune disease with genetic component of the Endocrine System; twenty five patients with cytological diagnosis and by mean of the determination of anti-thyroid antibodies of HAT were studied. Dosifications of TSH, T3, T4, antimicrosomal antibodies (AcM) and antithyroglobulin (AcT) were made before and after treatment as well as the determination of the goiter characteristics (size and consistency). Prednisone, 20 mg, was prescribed for 4 weeks; mean and percentage values were estimated. Results: It was observed that goiter decreased in 77,3% out the total, and a significant change was not observed in the consistency like "rubber". Mean values of AcM and AcT antibodies decreased significatively after treatment, but never reached normal levels; it was not observed change of TSH, T4 and T3 hormones after treatment. Conclusion: In spite of the treatment is not an specific one for theses diseases, immunosupressors could be used to decrease the glandular destruction resulting in hypothyroidism.

PIP44- GRAVE'S OPHTHALMOPATHY AND THE USE OF THE IMMUNOMODULATOR CYCLOPHOSPHAMIDE (ENDOXAN): OUR EXPERIENCE

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Grave's Ophthalmopathy appears in 50% of patients suffering from Graves-Basedow autoimmune disease and is caused by improperly immune response and excessive thyroid antibodies. Radiotherapy, steroids, decompression surgery and immune-modulators have been used as treatments. The Cuban experience with this therapy is rare or null; thus it motivated us to carry out this study in order to learn about the therapeutic effectiveness in treating this disease. **Material and Methods:** Twenty five (25) patients presenting Grave's Ophthalmopathy participated in the study, 15 (group 1) were administered 100mg of oral Ciclofusfamide during 6 weeks and 10 (group 2) daily oral steroids (prednisone 40mg) per 6 weeks. Titrating T4, T3, TSH pre and post-treatment and an Exophthalmometry before and after the treatment. **Results:** In group 1 the 87, 3% presented a marked diminution in the Exophthalmia measuring the degree with the exophthalmometer. The ocular paresis was expressed in 79, 5% of the total; the diplopia improved after the treatment in group 1. The values of T3 and T4 decreased post-treatment in group 2 significantly. **Conclusions:** The use of the immune-modulator Ciclofusfamide could be used as primary-line treatment in the complication of thyroid function.

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PIP45- CASE REPORT: USE OF THE HEBERBIOVAC-HB VACCINE AND HYPERIMMUNE GAMMAGLOBULIN IN AN INFANT BORN FROM A HBsAg-CARRIER MOTHER

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Introduction: Hepatitis B affects about 350 million people in the world, the most asymptomatic carriers. Risk groups include babies born from HBsAg-carrier mothers, other cohabitation with HBsAg carriers, drug-addicts, sexual promiscuity, health workers, and blood receptor individuals. Perinatal transmission is particularly important. For the prevention of the disease, it is important to achieve high and quick protective levels of antibodies; for that reason a combination between active and passive immunization is recommended. **Materials and Methods:** 0.5 mL (10 mcg) of Heberbiovac-HB (recombinant vaccine of HBsAg) was applied by intramuscular route in the anterolateral left thigh, and 1.0 mL of a Cuban anti-hepatitis B gammaglobulin by intramuscular route in the anterolateral right thigh. Both vaccine and gammaglobulin were applied at birth, at the first and the second months of life. The infant was again vaccinated at the first year of life. Blood samples for quantification of antibodies were taken at the eighth and thirteenth months of life. **Results:** Concentration of antibodies was 550 UI/mL at the eighth month of life, and 356 UI/mL at the thirteenth month of life. Adverse reactions were not detected. **Conclusions:** Proper immunity against hepatitis B was achieved after combination of the active and passive immunization in an infant born from HBsAg carrier mother.

PIP46- CELL AND HUMORAL IMMUNE RESPONSE IN IMMUNE DEPRESSED VACCINED PATIENTS WITH THE B ANTI HEPATITIS CUBAN VACCINE

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Hepatitis viral infection constitutes a world health problem. We have proposed to study the HEBERBIOVAC - HB Cuban vaccine specific inmunogenecity in inmunedepressed children likely to have immune response alterations. The main goal is to guarantee the right protection of our children against B hepatitis virus possible complications. Immunedepressed children who suffered from cell and humoral immunodeficiency were chosen from our medical service as well as healthy children as a group control with no antecedents of being infected with the virus and who completely accomplished the immunization scheme for the HEBERBIOVAC - HB vaccine. A control was performed at a year of the last dose to determine the antibodies titles so to know the retarded hyper sensibility response “in vivo” by a skin test as well as evaluating the cell immune response qualitatively which allows knowing whether they were adequately protected. It was shown that the immune depressed patients´ response was correct to the HERVEBIOVAC-HB vaccine and that a year later they also maintain the protection, although they always presented responses levels beneath the control group with significant statistics differences.

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PIP47- EVALUATION OF THE EFFECTIVENESS OF THE *Aloe barbadensis* EXTRACT FOR INYECTION IN INMUNO DEFICIENT PATIENTS

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The patient who has lesions caused by burns is an immune endangered patient; this situation propitiates the development of a syndrome of inflammatory systemic process which elevates the mortality of these patients. The hypothesis that the *Aloe barbadensis* extract could act as an inmunomodulator was demonstrated through out a stratified and controlled clinical trial. It was designed two groups: Group I received the normal therapeutics and Group II was administered the inyectable extract, besides the habitual medical treatment. The results showed a significant early reduction in the percentage of the CD_4^+ and CD_8^+ cells, nevertheless from the 10th day on, the patients who received the treatment with Aloe reached optimal results of these variables, similar condition was evident when the opsonophagocytic rate was evaluated, while the seric concentrations of the third component of the completion and of the immunoglobulins G, M and A on the sick patients of the Group II, reached the reference values on the third week of evolution. There were no adverse reactions attributable to the product used in the trial.

PIP48- EFFECTIVENESS OF INTACGL0BIN IN PATIENTS WITH IDIOPATIC THROMBOCYTHOPENIC PURPURA PREVIOUSLY TO TOTAL SPLENECTOMY

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Introduction: Patients with idiopathic thrombocytopenic purpura (ITP) have vulnerability to additional bleeding, them susceptible to severe hemorrhage. In patients with chronic ITP it may combine some treatments. The benefits of splenectomy probably combined effects of eliminating a source platelet antibody synthesis and the primary site of platelet destruction. The recent availability of intravenous gamma-globulin (IVIg) presents an opportunity to increase the platelet count previously to the surgical treatment. Objective: Study the response of treatment with IVIg in patients with chronic ITP previously to splenectomy. Material and Methods: This work that study 50 patients of the Hematology and Immunology Institute (HIT) with chronic ITP that received IVIg treatment previously to splenectomy, 25 pediatric patients and 25 adult patients. They received a dose of 400 mg/Kg/day during five days before to splenectomy. Results: In all patients we obtained a high rate of response and favorable duration. These results coincidence with several reports document the success in temporary platelet count elevation with IVIg. The more common side effects were fever, flushing and headache. All patients had been received immunosuppressive agents: with corticosteroids, Danazol and Athiotropine. In this study we use laparoscopy splenectomy in 13 patients and one patient need a second splenectomy for accessory spleen. Only 12 patients have relapse after of splenectomy. Conclusions: In contrast to other treatments the IVIg advantages inducing high rate of response. Considering that IVIg is one of the potential strategies to minimize the risk of severe thrombocytopenia in patients previously to surgical treatment.

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PIP49- THE IMMUNOGLOBULIN IGG THROUGH INTRAVENOUS ADMINISTRATION AS AN ADJUVANT THERAPY IN THE SEPSIS OF ELDER PATIENTS WITH ACUTE LEUKAEMIA

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The immunoglobulins are effector molecules of the immune humoral answer and their applications in suprafisiological doses in the control of the disregulation of the immune answer during the sepsis is related to their antimicrobian action by means of neutralizant and opsonizant antibodies against endotoxin or exotoxin bacterias, stimulating the production of reactive species of the oxygen, in leukocytes and the germicide activity of the serum and inhibiting the secretion of proinflamatory cytokines. (IL 1, IL 6, IL 18, TNF alpha). The sepsis constitutes a factor of bad prognosis in elder patients with acute leukaemia, so the antimicrobial therapy and the support measures together with the inmunomodulation are very useful. In our series of 37 to 60 year-old patients and over with acute leukaemia 23 sepsis episodes were presented post chemotherapy. The Intacglobin was used to 200 mg x Kg during 3 serial days as adjuvant therapy in 12 patients. The evolution was satisfactory in 8 of them with disappearance of the fever, in an average to the 5.2 days and stabilization of vital parameters, the most common adverse events were migraine and fever, evaluated as slight and possible causes, although they didn't modify the haematological parameters significantly in front of the group control, the number of cycles of used antibiotics and the period of hospitalization were diminished.

PIP50- IMMUNOSENESCENCE: FIRST MEASUREMENTS IN HEALTHY CUBANS

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Introduction: The immune system undergoes a wide range of changes with increasing age. Increased susceptibility to infections and reduced protection after vaccination reflect the impact of age-related changes on the immune system. In this study some phenotypic changes of lymphocytes were compared in Cubans healthy volunteers of different ages. **Materials and methods**: We employed 3 colour flow cytometry to asses lymphocyte subpopulations in a large sample of healthy donors (n=125). For analysis, the sample was divided in 3 groups according to the age: young, old and very old (less than 60, more than 60 and more than 80 years old, respectively). **Results**: Changes in the number of human Lymphocytes were associated to the aging process. Our study showed that B lymphocytes counts decrease with age. Furthermore we found an increase in both T $CD4^+$ numbers with a terminal differentiate phenotype and the percentages of $CD8^+CD57^+$ T cells in elderly subjects. Moreover, there is an inverse correlation between the percentages of CD19+ B cells and the number of differentiated T cell subsets. In addition, there are gender differences in CD4 and CD8 terminal differentiated cells counts. The high number of these cell subsets correlated directly with age in men. **Conclusions**: The immune system is affected by the aging process, at the level of the effector T-cells and B lymphocytes. All these changes could have a negative impact on the immune response of elderly patients during any type of immunotherapy.

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PIP51- IMMUNOTHERAPY IN BREAST, COLON AND OESOPHAGUS CANCER: MANAGEMENT OF CLINICAL TRIALS IN SANTIAGO DE CUBA

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The therapeutic of the patient with cancer has enhanced its possibilities with the immunotherapy. This therapy can be used as a complement to conventional treatments such as surgery, x-ray and chemotherapy. The therapeutic vaccines against cancer constitute a new immunotherapeutic strategy that stimulates in the patient the immune response against tumour antigens. So far, many vaccines types exist and they are tested in different locations. The subcentre of clinical trials of Santiago de Cuba has a vast experience in the management of these studies; that's why in the present work we will offer a panoramic of these investigations in patients with breast, colon and oesophagus cancer. Additionally, the quality of trials related to Good Clinical Practices fulfilment will be evaluated. Two hospitals that execute 6 national multi centric trials (phase I and II) were analysed. The vaccines 1E10, HR3 and N-Glicolil from Molecular Immunology Centre were the selected products. The Investigator folder, Consents, Clinical Histories, Collection data Notebooks and visit reports were the reviewed documents. Results showed that from 85 patients only 21 fulfilled the inclusion criteria. It was proved the necessity to continue with the qualification of searchers in Good Clinical Practices. In fact, it will have an influence on the safe use of products and the excellence of medical assistance.

PIP52- USE OF IMMUNOTHERAPEUTIC PRODUCTS IN CLINICAL ASSAYS PERFORMED IN SANTIAGO DE CUBA. AN OVERVIEW

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During last three decades the therapeutic treatment of patients with cancer has enhanced its possibilities with the incorporation of a fourth modality denominated Immunotherapy. This therapy can be used to complement some of conventional oncospecific treatments, and it is based on focusing the immune response against tumour cells to obtain a repair, stimulation or amplification of responsible immune mechanisms involved in tumour's growth and dissemination. This work summarizes the results obtained during 15 years in the management of clinical trials performed with immunotherapeutic products in Santiago de Cuba. In these years, more than 100 investigators of 22 medical specialities and 4 of our institutions (Hospital Oncológico Conrado Benítez, Saturnino Lora, Juan Bruno Zayas and Infantil Sur) have participated in clinical trials. Additionally, we have executed 18 protocols in 15 localizations such as prostate, glyoma, lung, esophagus, colon, ovary, skin by using 8 immunotherapeutic products from the Molecular Immunology and Genetic Engineering Centres, so around 170 patients have been beneficiated. Overall, all investigations have been performed respecting the ethical principles and good clinical practices.

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PIP53- THE INMUNOGENICITY AND REACTOGENICITY OF THE QUIMIOHIB CUBAN VACCIN IN UNDER ONE- YEAR-OLD HEALTHY INFANTS

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The infections by *Haemophilus influenzae* tipe b (Hib) are very frequent in paediatric ages, causing serious infections leading to death. When the application of the Cuban vaccine against this germ began, we carried out a longitudinal, prospective study with the healthy breastfed children of the Policlinic "José Antonio Echeverría", Cardenas, to determine the immunogenicity and reactogenecity of this vaccine. Our universe was the children born between September 1 st and October 31 st , 2004, taking as sample only 32 healthy breastfed children. To select them we applied criteria of inclusion and exclusion; to the recruited children, with the previous informed consent of their parents, were applied three doses of the Cuban vaccine QuimiHib®, following the scheme 2-4-6 months, intramuscular way, on the anterior side of the right thigh. A specialist in paediatrics and the vaccinating nurse rigorously followed the adverse events taken place during the three consecutive days after vaccination, and during the 7-to-30 days period after the administration of each doses. We calculated the percentages of presentation of these events by levels, from moderated to severe. We determined at what time they appeared more frequently after vaccination, and we evaluated the immunogenicity of the product at the 30 th day of finishing the complete series of immunization by determination the titles of IgG antibodies against Hib. The adverse reactions, most of them insubstantial, were solved without applying drugs. All the children coming to the end of the treatment acquired the required level of antibodies.

PIP54- IMMUNOMODULATOR'S PRESCRIPTION IN NEONATES WITH SEPSIS AT THE HOSPITAL INFANTIL NORTE OF SANTIAGO DE CUBA

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Introduction: Since the sepsis birth mortality continues to be high in spite of the big advances in the taken care of intensive birth and the use of wide-range antibiotics, they are coming in for new therapeutic their strategies that they improve survival, among the ones that find the immunogenic therapeutics. We decide that evaluating immune modulator's prescription in neonates with sepsis in Neonatology's service of Hospital Infantil Norte of Santiago de Cuba from January 2006 to December 2007.**Material and methods**: Was realized a descriptive and retrospective study of use of drugs of the type prescription-indication with elements of therapeutic scheme and results of immune modulator's use. The sample was conformed for 56 patients, the same one was evaluated using clinics and humoral variables. **Results**: Therapy was mainly use through Intaglobín for a 85,7 % and Transference Factor for a 14,3 %, indicating that there was predominance of adequate prescription (73.2 %) , totally difference with the inadequate ones (26.7 %) out of the 56 patients, the 96,43 % left the hospital alive and in the 94,8 % there was favourable humoral variation in relation to the leucocytes formula before and after the use of this therapy. **Conclusions**: Of the total of prescriptions evaluated, the adequate prevailed. In regards to be non satisfactory answers be adequate ones prevailed, long stay at the Hospital prevailed in regard to the short ones with predominance of alive patients. They did not yield adverse reactions in the clinical histories revised.

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P55- ABDOMINAL INFECTION, IMMUNE RESPONSE. REVIEW OF THE BIOLOGICAL, MOLECULAR, PHYSIOLOGICAL, AND CLINICAL ALTERATIONS

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Recent progress in molecular biology has made possible to identify the basic mechanisms by which bacterial components interact with the innate immune system to activate the inflammatory response. We propose a review of the biological, molecular, physiological, clinical alterations they produce was made, making emphasis on their immunodepressive effect and differentiating the inflammatory response to sepsis known among the most severe patients as uncontrolled and hypertensive immunohumoral response or malignant inflammatory response. The role played by the mediators of inflamation in itself, as well as the ways to fight immunodepression are approached. It was concluded that the decrease of the immune response may be measured, prevented and corrected, or at least modified with drugs and supplementary nutritional support.

PIP56- IMMUNITY AND FERTILITY - LIF GENE MUTATIONS IN WOMEN DIAGNOSED WITH UNEXPLAINED INFERTILITY AND ENDOMETRIOSIS HAVE NEGATIVE IMPACT ON THE IVF OUTCOME

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The frequency of functionally relevant mutations of the leukemia inhibitory factor (*LIF)* gene in infertile women is significantly enhanced in comparison with fertile controls. The objective of this retrospective cohort study was to evaluate the impact of the lif gene mutations on the outcome of the treatment in women with various causes of infertility. Fifteen infertile women with functional lif gene mutation, the G to A transversion at the position 3400 leading to the valin to methionin exchange at codon 64 were analyzed. Group A was formed by women with diagnoses that are frequently accompanied by changes in humoral as well as cell-mediated immunity - idiopathic infertility (n=4) and endometriosis (n=3). Group B comprised of patients with PCOS (n=3), andrologic factor (n=3), tubal factor (n=1) and hyperprolactinemia (n=1). Control group comprised of 136 infertile women with no lif gene mutation. Seven of the mutation-positive patients were successfully treated by first cycle of in vitro fertilization (IVF) but in this group there was nobody diagnosed with idiopathic infertility and only one with endometriosis which means that there is a statistically significant difference in the pregnancy rates between groups A and B (P=0.029, Fisher´s 2 by 2 Exact test) and between the group A and control population. The results suggest that the success of the infertility treatment in mutation-positive women is influenced by the cause of infertility - idiopathic infertility and endometriosis have negative impact on the IVF outcome. This study was supported by the IGA MZd NR/ 9135-3.

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PIP57- PRESCRIPTION OF THE CUBAN MONOCLONAL ANTIBODY IOR-T3 IN PATIENTS WITH STEROID-RESISTANT ACUTE REJECTION IN KIDNEY TANSPLANTATION

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Introduction: The implant acute rejection continues being the main barrier that intervenes to the success of organs transplant and it appears when the basal immunosuppressor treatment fails. The metilprednisolona administered in form of pulses is the drug of first line for the treatment of the implant acute rejection. When the steroids fail this rejection is considered resistant and then drugs of second lines are used, as the Cuban monoclonal antibody ior-T3. **Objective**: To evaluate the prescription of the Cuban monoclonal antibody ior-T3 in patients with steroid-resistant acute rejection in kidney tansplantation. **Materials & Methods**: A pharmacological study in 25 patients with steroid-resistant acute rejection and renal transplant were carried out. They were diagnosed according to clinical, humoral and histological criteria and prescribed the Cuban monoclonal antibody ior-T3. The patients were continued regularly in external consult during two years after treatment. **Results**: It was observed that 68 % of the cases showed a suitable answer to treatment. The implants were normofunctionant after two years. In 40.9 % of the patients adverse reactions were presented, as fever, chills and diarrhea. **Conclusions**: It was concluded that the patients had a good answer to treatment. The entirety of the prescriptions was adequated.

PIP58- EVEROLIMUS, AN APPROACH TO ITS EFFICIENCY IN RENAL TRANSPLANT

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Introduction: The transplant of organs continues being the election treatment for many patients. The progressive dysfunction of the implant continues being an essential problem for the long term survival. The everolimus associated with ciclosporin (CsN) possesses good effectiveness. In this research we wonder if it is more efficient for the society, the treatment with everolimus and reduced dose of CsN in front of the mycophenolate with full dose of CsN, in the countries of Central America and the Caribbean. **Materials & Methods:** We present a cost-effectiveness study that compared 5 alternatives treatments with the habitual of mycophenolate + full dose of CsN, combinations of different dose of everolimus + reduced dose CsN and adding basiliximab. We considered the cost of the medications, those derived of the pharmacological treatment of the adverse drug reaction (ADR) and relatives of laboratories test. The evaluated outcomes were the lack of effectiveness (defined as acute rejection confirmed by biopsy, the implant´s lost, death or follow up lost) and the creatinin levels. 5 clinical researches were reviewed and we used the 2003´s costs in USD dollars. **Results:** The use of 0.75 mg of everolimus, bid + reduced dose CsN was as effective as the Mycophenolate mofetil + full dose of CsN, but less expensive ($25.154,68 vs. $49.603,00). Adding basiliximab turned out the most efficient (83, 8%) and the alternative with better cost-effectiveness relationship (C/E = $287, 35). **Conclusions:**: Although everolimus´s use has the highest costs for adverse and complementary reactions, it decreases the costs of the immunosuppressant treatment, being more efficient and more effective than the habitual treatment, in dose of 1.5mg/day adding basiliximab. The most frequent adverse event reported with the everolimus was hyperlipidaemia for that reason its safety need to be monitored.

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PIP59- IMMUNE MECHANISM OF THE PRINCIPLES HIPERSENSITIVITY ADVERSE DRUG REACTIONS REPORTED TO THE CUBAN SURVEILLANCE SYSTEM

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Introduction: Adverse drug reactions can cause negative patient outcomes, increase healthcare utilization, and contribute to rising healthcare costs. From the pharmacological point of view, hypersensitivity reactions are common. The Cuban Drug Surveillance System has records of 5 years of following adverse events in the market. **Materials & Methods**: We present a descriptive study, based on the spontaneous adverse event reporting method. During 5 years we collected the adverse drug reactions under the hypersensitivity terms (skin rashes, anaphylactic reactions, urticaria and others) in the national data base. The adverse effects were classified according to severity, frequency and pharmacological mechanism. **Results:** In five years, hypersensitivity reactions took the second place of reported reactions after gastrointestinal effects. Anaphylactic reactions (hypersensitivity I) were the principal mechanism advocated, followed by citotoxic reactions (hypersensitivity II). The Epidermal Toxic Necrolisis and Steven Johnson Syndrome were one of the most severe conditions reported to our system with this kind of pharmacological mechanism. **Conclusions**: Hypersensitivity mechanism is frequent in the Cuban System and there are commonly severe and unpredictable.

PIP60- HYPERSENSITIVITY REACTIONS ARE FREQUENT IN CUBA? DATA FROM THE DRUG SURVEILLANCE CUBAN PROGRAMME

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Introduction: Adverse drug reactions (ADRs) are any noxious, unintended, or undesired effects of a drug that occur at doses used in humans for prophylaxis, diagnosis, or treatment. The Cuban Drug Surveillance System has records of 5 years of following adverse events caused by drugs, biological, vaccines and natural products and several analyses has been made to improve the sensitivity and quality of the system. **Materials & Methods**: We present a descriptive study, based on the spontaneous adverse event reporting method. From years 2003 to 2007 we collected the adverse drug reactions under the hypersensitivity terms (skin rashes, anaphylactic reactions, urticaria and others) in the national data base. The adverse effects were classified according to drug related, severity, frequency and causality assessment. **Results**: In five years, hypersensitivity reactions took the second place of the totality of reported reactions. The drugs more related were Antibiotics (Penicillin, Cephalosporin, Sulphas), Anti-inflammatory drugs (Ibuprofen, Dypirone, Aspirin), and others. Anaphylactic reactions (hypersensitivity I), followed by citotoxic reactions and Epidermal Toxic Necrolisis and Steven Johnson Syndrome were the most severe conditions reported to our system. Most of these reactions were moderate and severe and probable. **Conclusions**: Hypersensitivity mechanism is frequent in the Cuban System. A lot of different drugs and products can produce them and there are commonly severe, probable and unpredictable.

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PIP61- DETECTION OF POSITIVE SELECTION IN COMPLETE GENOMES OF HUMAN PATHOGENS

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The high antigenic diversity observed in the human pathogens has evolved as strategy for evading immune attack. It is effective against both natural and artificially induced immunity, and represents a major obstacle to the development of vaccines against pathogens like *Neisseria meningitidis*, hepatitis C virus (VHC), among others. The necessity to find and to apply methods that allow to identify these antigens as well as to locate specific regions that can be under strong positive selection which can contribute to the variability, it becomes evident. Generally the investigations carried out with the objective of detecting the presence of positive Darwinian selection in proteins, have been focused toward the study of punctual cases. However we could be in presence of a complex system of evolutionary adaptation that takes into account a bigger number of proteins that are not even recognized as potential antigens. In this work a method was developed with the purpose of detecting regions where signs of the positive selection is present, in complete genomes of human pathogens. With the analysis of well characterized 6 genomes, was possible to identify regions under evolutionary adaptation. Some identified regions were previously reported by other investigators. It was interesting that other regions constituted potential virulence factors or presented neutral or conservative signal selections mainly in proteins that are integrated in the outer membrane vesicles used in the Cuban vaccine against *N. meningitides*, named VA-MENGOC-BC®.

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POSTERS (P)

MONDAY, APRIL 21

Inflammation and Pain (IF)

Chairs: Pedro Camilo Rodriguez, Eva Marrero, Gilberto Pardo, Grisel del Toro, Maria Acelia Marrero, Beatriz Garrido (Cuba)

PIF01- ROLE OF iNOS-DERIVED NITRIC OXIDE IN INDOMETHACIN-INDUCED INTESTINAL DAMAGE

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NSAID-induced gastrointestinal ulcers constitute one of the main adverse reactions associated to pharmacological treatment. Alterations of epithelial and vascular architecture as well inflammation are characteristics of this kind of damage. Nitric oxide (NO) is a ubiquitous biological mediator involved in the regulation of different cellular functions. In the present study we analyze the role of iNOS-derived nitric oxide in the evolution of intestinal damage induced by indomethacin and their ability to modulate epithelial function through the expression of intestinal trefoil factor (TFF-3), a peptide involved in mechanisms of defence and repair. Methods: Four group of Sprague-Dawley male rats were established which received subcutaneously indomethacin (7.5 mg/kg) or vehicle (HCO_3^- 5%). The other two groups received 1400 W, 5mg/kg, an iNOS inhibitor, before the treatment with indomethacin or vehicle. We analyze macroscopic damage in duodenum, jejunum and ileum taking into accounts a score (1-8) which consider the presence of eritema, ulcers and intestinal walls adhesion. After it, the expression of iNOS and TFF-3 (quantitative RT-PCR and western blot) in the three intestinal segments was determined. Results: Considering the score, indomethacin induced damage in duodenum (3±1), jejunum (6±0.1) and ileum (5.5±0.5). In the other hand, it enhanced the iNOS mRNA in all of these segment and iNOS inhibition significantly prevented macroscopic damage with a mean value of 1±0.1 in the three areas ($p<0.05$). In control animals, 1400W did not induce any apparent macroscopic damage. In the duodenum of indomethacin treated rats the expression of TFF-3 mRNA and protein was increased 3±0.1 and 1.8±0.3 fold, respectively compared with that observed in control rats. The increase in the amount of protein was significantly avoided (0.9±0.05) in the duodenum of animals receiving 1400W. In jejunum and ileum of indomethacin-treated rats, an increase in mRNA and protein for TFF-3 is also observed and preliminary results suggest that such an increase is prevented in 1400+indomethacin-treated rats. Conclusions: Indomethacin-induced intestinal damage is mediated by iNOS-derived NO synthesis and it is associated with an induction in TFF-3 expression. NO may play a dual role in the evolution of intestinal damage: it would act as an ethiopathogenic agent in the initial phase of damage and it would trigger the expression of TFF-3 in the late stage.

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PIF02- THE EFFECT OF iNOS INHIBITORS TREATMENT IN EXPERIMENTAL COLITIS MODELS

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Introduction: Our aim was to investigate the effectiveness of amino guanidine (AMG), an iNOS inhibitor treatment on experimental colitis model, resembling ulcerative colitis. **Materials & Methods**: We induced colitis by applying 4% acetic acid (AA) in transrectal route to 18 Sprague-Dawley rats, weighing 200-300 gr. Rats were divided into 2 groups. In control group (n=9), we applied 2 ml. serum physiologic in intraperitoneal route daily for seven days. In AMG group (n=9), 2 hours after induction, 100 mg/kg AMG was applied intraperitoneally in two hours twice a day, for seven days. At the end of 7 days, all the rats were sacrified and the distal 10 cm part of colon were examined macroscopically and scored. In microscopic examination, edema, inflammation, ulceration, crypt hyperplasia, crypt distortion and loss of goblet cells were scored. NO levels in urine were measured by Griess method. **Results**: The histological findings, showing the severity of colitis were reduced by AMG, application. The improvement in histological findings were significant in AMG group comparing the control group($p= 0.001$). Weight loss was significantly lower in AMG ($p<0.001$). In colitis induced by AA, we showed that nitric oxide activities were depressed by AMG ($p<0.001$). **Conclusions**: In rats, colitis induced by AA, treatment reduced weight loss and colonic inflammation and depressed NO levels.

PIF03- ANTIINFLAMMATORY EFFECTS OF SYNTHETIC 1-O-NONANYLGLYCEROL IN ADYUVANT-INDUCED CHRONIC INFLAMMATION MODEL

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The angiogenesis is an important morphogenetic process involving the growth of new blood vessels from pre-existing vessels. The chronic inflammatory response use the angiogenesis to destroy inflammatory tissue.[1,2] The 1-O-alkylglycerols are structural analogs of ether lipids and considerate modifiers of the biological response.[3] Previously studies showed that natural and synthetic ethers with large chain and pair number of carbon atoms (12, 16 and 18) have anti-inflammatory and antiangiogenic effects.[4-6] In this work is present the *in vivo* anti-inflammatory effect of synthetic 1-O-nonanylglycerol (C9) on angiogenesis in adjuvant-induced chronic inflammation model (Kobayashi y col., 1998).[7] Spreague Dawley rats were administrated (i.p. injection) with 20 and 40 mg/kg body weight using hydrocortisone (10 mg/kg b.w.) as positive control. They were measured the serum concentration of acute phase protein (C reactive protein, C3, C4, haptoglobin), the extension of neovascularization, the weight (g) of granuloma and its red carmine concentration. Was performed a histopathologic study of tissue. The results revealed the usefulness of the model to obtain a good established granuloma at 6 days in the negative control group. C9 (20 and 40 mg/kg) inhibited both the formation of granuloma and the associated neovascularization with a significantly decrease on its weight and the carmine concentration respectively. It showed a doses-dependent effect. The histopathologic study corroborated these results. In the other way it significantly decrease the serum concentration of C reactive protein, one of the more sensible phase acute reactant.[8]

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Synthetic 1-O-nonanylglycerol like the previously studied alkylglycerols have anti-inflammatory and antiangiogenic effects. References: 1- Larsen and Henson (1983). Mediators of inflammation 1: 355-359. 2- Folkman J. (1985). Perspect Biol Med 29: 10-36. 3- Brohult et al. (1970). Acta Chem. Scand. 24: 730. 4- Bilbao et al. (1998). Memorias Joven Ciencia: 115. 5- Sotomayor H. (1999). [Tesis de Maestría]. La Habana: Universidad de La Habana. 6- Sotomayor et al. (2005). Bioquimia 30 (1): 5-12. 6- Kobayashi et al. (1998). Biol Pharm Bull 21 (4): 346-349. 8- Golsdby et al. (2000). Kuby Immunology. Fourth edition. Ed. Freeman.

PIF04- SYNTHETIC 1-O-UNDECYLGLYCEROL: *in vivo* ANTIINFLAMMATORY ACTION

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The 1-O-alkylglycerols, structural analogs of ether lipids, are considerate modifiers of the biological response.[1] Natural and synthetic ethers with large chain and pair number of carbon atoms (12, 16 and 18)[2-5] have anti-inflammatory and antiangiogenic effects. This work describes for firs time the *in vivo* anti-inflammatory effect of synthetic 1-O-undecylglycerol (C11) on angiogenesis in adjuvant-induced chronic inflammation model (Kobayashi y col., 1998).[6] Spreague Dawley rats were administrated (i.p. injection) with 20 and 40 mg/kg body weight of C11 using hydrocortisone (10 mg/kg b.w.) as positive control. It was visualized the extension of neovascularization, it was measured the serum concentration of acute phase protein (C reactive protein, C3, C4, haptoglobin), the weight (g) of granuloma and its red carmine concentration. Was performed a histopathologic study of tissue. The results revealed the usefulness of the model to obtain a good established granuloma at 6 days in the negative control group. There were difference between negative control and treated groups. C11 (20 and 40 mg/kg) inhibited both the formation of granuloma and associated neovascularization with a significantly decrease on weight and the carmine concentration respectively. It showed a dose-dependent effect. The histopathologic study corroborated these results. In the other way it significantly decreases the serum concentration of C3 and C reactive protein, one of the more sensible phase acute reactant.[7-8] Synthetic 1-O-undecylglycerol like the previously studied alkylglycerols has anti-inflammatory and antiangiogenic effects. References: 1- Braquet P. (1988). Prog Biochem Pharmacol 22: 46-57. 2- Brohult et al. (1970). Acta Chem. Scand. 24: 730. 3- Bilbao et al. (1998). Memorias Joven Ciencia: 115. 4- Sotomayor H. (1999). [Tesis de Maestría]. La Habana: Universidad de La Habana. 4-Sotomayor et al. (2005). Bioquimia 30 (1): 5-12. 5- Kobayashi S, et al. (1998). Biol Pharm Bull 21 (4): 346-349. 6- Larsen and Henson (1983). Mediators of inflammation 1: 355-359. 7- Folkman J. (1985).. Perspect Biol Med 29: 10-36. 8- Golsdby et al. (2000). Kuby Immunology. Fourth edition. Ed. Freeman.

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PIF05- EFFECTS OF MELATONIN, FISH OIL AND VITAMIN E ON THE CYCLOOXYGENASE-2 ACTIVITY AND OXIDATIVE STRESS IN MIDDLE BRAIN OF C57/BL6 MICE AFTER THE ADMINISTRATION OF 1-METHYL-4-PHENYL-1, 2,3,6 - TETRAHYDROPYRIDINE (MPTP)

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Introduction: Parkinson's disease (PD) is a neurodegenerative disorder of unknown pathogenesis which is characterized by the loss of nigrostriatal dopaminergic neurons, increased cyclooxygenase-2 (COX-2) activity and the production of prostaglandin E2 (PGE2). This study determined the effect of fish oil, melatonin and vitamin E in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease (PD). **Materials and Methods:** Mice of the C57/BL6 strain were used. Control groups were treated with Melatonin; Fish oil Vitamin E and physiologic saline solution, respectively. Four experimental groups were: MPTP; MPTP plus Melatonin; MPTP plus Fish oil and MPTP plus Vitamin E. Experiments were conducted in acute phase (6 and 24 hours)) and chronic phase (7 and 15 days) after administration of MPTP. We evaluated the: a) COX-2 activity b) lipid peroxidation products (malonadialdehide and 4-Hydroxyalkenals); c) nitric oxide catabolites (nitrate/nitrite). **Results:** The increased COX-2 activity induced by MPTP was inhibited especially, with fish oil (Melatonin and vitamin E also showed inhibition). In addition, oxidative stress induced by MPTP was significantly decreased by melatonin. **Conclusion:** Fish oil rich in EPA inhibits the activity of cyclooxygenase-2 and the oxidative stress induced by MPTP. In these experiments the higher effect was found with Melatonin. Acknowledgments: ZoneDiet, México; Dra. Silvia Orozco Aviña & Dr. Gustavo Orozco Aviña.

PIF06- SESQUITERPENE LACTONE FRACTION FROM *Artemisia khorassanica* INHIBITS iNOS AND COX-II EXPRESSION THROUGH THE INACTIVATION OF NF-κB

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Introduction: The genus *Artemisia* L. is one of the largest and most widely distributed of the Astraceae (Compositae) in the world and *Artemisia khorassanica* is endemic to Iran. Members of the *Artemisia* genus are important medicinal plants throughout the world. **Materials and Methods:** The present study focuses on the effects of sesquiterpene lactone fraction from *Artemisia khorassanica* (SLAK) on lipopolysaccharide (LPS)-induced nitric oxide (NO), prostaglandin E_2 (PGE_2), tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) production in the mouse macrophage J774A.1 cells. Moreover, we evaluated SLAK modulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) enzyme expression by western blot analysis. **Results:** Our data revealed that SLAK (10-200 µg/ml) in a dose-dependent manner inhibits NO, PGE_2, TNF-α and IL-1β production induced by LPS in the J774A.1 cells. These data were consistent with the modulation of iNOS and

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COX-2 expressions. It was also showed that SLAK suppresses the iNOS and COX-2 enzyme expression through the inhibition of NF-κB activity. **Conclusions:** This study is the first report of anti-inflammatory effect of *Artemisia khorassanica*. Our results demonstrated that *Artemisia khorassanica* from Khorasan Province could be good candidates for the investigation of anti-inflammatory activity *in vivo*. So, isolation of effective compounds in this fraction and elucidation of their structures will be essential.

PIF07- INHIBITION OF INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION BY THE KOPETDAGHINS FROM *Dorema kopetdaghense* IN J774A.1 MACROPHAGES

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Introduction: Nitric oxide (NO) is synthesized in large quantities by activated inflammatory cells and has been demonstrated to be involved in the pathogenesis of acute and chronic inflammatory conditions. **Materials and Methods:** We investigated the effects of sesquiterpene derivatives isolated from *Dorema kopetdaghense* (kopetdaghins A, C, E) on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in the mouse macrophage J774A.1 cells. In addition, the effect of these compounds on cell viability and inducible nitric oxide synthase (iNOS) expression was evaluated. **Results:** The results of our study showed that kopetdaghins (10-100 µg/ml) significantly inhibited the enhanced production of NO induced by LPS in a dose dependent manner in the J774A.1 cells. Treatment with kopetdaghins did not reduce cell viability at any dose used. Treatment of macrophages with kopetdaghins also caused a significant inhibition of iNOS expression. These observations suggest that the inhibition of NO production by kopetdaghins may be due to its inhibitory effects on iNOS expression. **Conclusions:** This study suggests that kopetdaghins has significant anti-inflammatory activity and has potential for the treatment of inflammatory diseases. This study is the first report of anti-inflammatory effect of the sesquiterpenes of *Dorema kopetdaghense*.

PIF08- ANTINFLAMMATORY EFFECTS EVALUATION OF A CARBOHYDRATE OF *Bromelia pinguin* USING THREE NON-CLINICAL MODELS

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Antinflammatory activity of a carbohydrate of Bromelia pinguin using three inflammation models is carried out. Carragenin plantar oedema (E-1):mice (25 to 30 g BW), 5 groups, 6 animals each, dosed: 25, 50 and 100 mg/kg BW, positive control: indomethacin and control vehicle (distilled water) orally . Later carragenin 1% (0,5 ml) was injected in the posterior right plantar region, measuring thickness (0, 1, 2, 3, 5 and 7 hours) calculating the inflammatory reaction inhibition percent. Ear croton oil oedema (E-2): male mice (20 g BW) 2 groups, 6 animals. Irritant croton oil (5%) topically applied in the right pavilion ear (10 µl), control group: no treatment, then 0,5 mg/kg of the substance was orally applied; after 30 min ear pavilion discs were taken, weighed to calculate: inflammation and inflammation inhibition percents. Croton subcutaneous granulom (E-3): male rats (150 to 200g BW), 5 groups, 5 animals each. Two cotton pellets were subcutaneously placed (scapular region), 7 days dosed:

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25, 50 and 100 mg/kg of the substance, the next day animals were extracted granuloms (humid and dried weighed) calculating chronic inflammation activity. Results: E-1: 25 mg/kg was similar to negative control, 50 mg/kg: indomethacin comparable activity (hours 1 and 2), 100 mg/kg: similar to indomethacin and sustained in time. E-2: Negative control: 14,06 % inflammation and treated groups 11,76%, no statistical differences. E-3: comparing treated to negative control: no statistical differences, contrarialy compared to positive control, indicating no chronic antinflammatory action. E- 1 showed moderated antinflammatory activity and no antinflammatory activity in E-2 and E-3.

PIF09- BONE PROTECTIVE EFFECTS OF D-003 IN EXPERIMENTAL MODELS OF PRIMARY AND SECONDARY OSTEOPOROSIS

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Increased osteoclast activity respect to osteoblast function is pivotal in osteoporosis development. Mevalonate-to-cholesterol pathway is key for the production of the isoprenoids required for protein prenylation, a process essential for osteoclast activity, and increased lipid oxidation has been linked to osteoporosis. The inhibition of both mevalonate-to-cholesterol pathway, and lipid peroxidation (LP) can contribute to prevent the bones from osteoporosis. Sugarcane wax intake has shown to inhibit osteoporosis in rats fed a carbohydrate and oil restricted diet. D-003 is a reproducible mixture of high molecular weight acids purified from sugarcane wax, wherein octacosanoic (C_{28}), triacontanoic (C_{30}), dotriacontanoic (C_{32}), and tetratriacontanoic (C_{34}) acids are the most abundant and C_{24}-C_{27}, C_{29}, C_{31}, C_{33}, C_{35}, C_{36} acids are at lower concentrations. D-003 has shown to inhibit cholesterol synthesis prior to mevalonate formation and to prevent LP. Oral treatment with D-003 (5 – 200 mg/kg) administered for 3 months has shown to prevent the increased bone loss and bone resorption in ovariectomized (ovx) rats by increasing osteoclast apoptosis in a dose-dependent manner. The magnitude of these effects of D-003 has been comparable to those of alendronate, estrogens and pravastatin. The bone protective effects of D-003 on ovx rats have been persistent after 12 months of treatment. Also, D-003 (5 – 200 mg/kg) given for short-term has prevented bone loss and bone resorption in rats with glucocorticoid-induced osteoporosis. We hypothesize that these bone protective effects of D-003 could be linked to its inhibitory effects on the mevalonate-to-cholesterol pathway, as occurs with bisphosphonates and statins, and to its antioxidant effects.

PIF10- PRECLINICAL VALUTATION OF THE ANTIINFLAMMATORY ACTIVITY OF WHITE BIDENS (ROMERILLO)

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The use of the herbs goes back to the oldest origins of the humanity. The past century arose a vivid interest by the study and consumption of the herbs which opened a wide field for their application. White Bidens is well known in our country as romerillo and has been used in the treatment of different affections and has inquired into its anti-inflammatory properties that motivated the development of this investigation. An experimental study was carried out with the objetive to determine the anti-inflammatory effect. 30 Sprague Dawley male rats, 30 rats to wistar male rats and 30 Swiss mice were used determination of the anti-inflammatory activity by means of the technique of induced granuloma by cotton discs, plantar edema by carrageenan and auricular edema by oil of croton. The extract was used by oral route in three different

doses and its effect was compared with alcohol 50 % (vehicle of the extract), indomethacine and bicarbonate to 4 %. the extract of administered white Bidens in dose of 200mg/kg showed to comparable action anti-inflammatory with the obtained one with indomethacine in the technique of granuloma induced by cotton discs. This extract is as effective as the indomethacine in the protection of edema to plant induced by carrageenan. The prepared one of romerillo (200 mg/kg) inhibits in addition the inflammatory answer caused by the oil administration of croton. Although the effect anti-inflammatory of white Bidens is not superior to the reached one with the indomethacine, can suggest the use of the plant in those cases in that it is desired to avoid the adverse reactions of this type of drugs.

PIF11- ANTI-INFLAMMATORY EFFECTS OF *Musa paradisiaca L* EXTRACT (Acitan®)

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Musa paradisiacal L is a plant of the family Musaceae. The parts different of this plant (fruit, pulp, leaves) has been used as a folk medicine for treatment of peptic ulcers, analgesic, antiasmatic. Chemical constituents of the extract have been investigated, and some of them identified (tannins and phenols). In the present study we investigated effects of the extract on anti-inflammatory mechanism in a rat model of subplantar oedema and ulcerative colitis induced by acetic acid. Methods: For subplantar oedema female Wistar rats were divided into five groups: Acitan groups (54, 64, 74 mg/kg respectively p.o), indomethacin group and control group. Oedema was induced by subplantar administration of carragenan. Phospholipase A_2 levels were evaluated in sample of blood. For ulcerative colitis female Wistar rats were divided into tree groups: Acitan group, Azulfidine group as a positive control group and acetic acid control. Colitis was induced by intracolonic administration of 3% acetic acid. Colonic inflammation was evaluated by colonic myeloperoxidase (MPO) activity, macroscopical mucosa evaluation, weight of colon, mastocites degranulation in colonic mucosa. Results: Compared to the control group, both Acitan and Indomethacin showed a significant antinflammatory effect of oedema subplantar ($p<0.05$) the ED50 estimated was 57, 54 mg/kg. The treatment with Acitan decreased colonic concentrations of MPO, additionally, levels mastocites degranulation in colonic mucosa was significantly lower in the Acitan group and Azulfidine group than in the acetic acid group. Conclusion: Acitan could reduce inflammatory injury in rat models of subplantar oedema and ulcerative colitis.

PIF12- ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF EXTRACTS FROM THE STEM BARK OF *Croton macrostachyus* (EUPHORBIACEAE) IN RATS AND MICE

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The present study evaluates the antinociceptive and anti-inflammatory properties of the aqueous and methylene chloride/methanol (CH_2Cl_2/CH_3OH) extracts of the stem bark of *Croton macrostachyus* (Euphorbiaceae). The extracts administered orally at the doses of 150, 300 and 600 mg/kg were examined on pain induced by acetic acid, formalin and pressure and on inflammation induced by carragenan, histamine and formalin-induced

inflammation. Both extracts induced a significant dose-dependent ($P < 0.001$) reduction in the number of abdominal constrictions induced by acetic acid with a percentage of inhibition ranging from 29.88 to 57.77 for the aqueous extract and from 40.90 % to 73.33 % for CH_2Cl_2/CH_3OH extract. The three doses of the two extracts also significantly reduced ($P < 0.001$) the two phases of pain induced by formalin, with maximum inhibition of 76.27 % and 91.55 % provoked at the first and second phases respectively by the CH_2Cl_2/CH_3OH extract at 600 mg/kg. At the same dose, the aqueous and the CH_2Cl_2/CH_3OH extracts inhibited the pressure induced pain by 54.39 % and 61.31 % respectively. The two extracts exhibited anti-inflammatory activity, the CH_2Cl_2/CH_3OH extract being the most active, inhibiting the acute inflammation induced by carrageenan, histamine and formalin by 58.82 %, 55.13 % and 41.97 % respectively. Both extracts also significantly reduced the chronic inflammation induced by formalin. These results show that the aqueous and CH_2Cl_2/CH_3OH extracts of the stem bark of *Croton macrostachyus* possess analgesic and anti-inflammatory properties that may result from their peripheral and central effects. These findings are in accordance with the traditional use of the plant and indicate that *Croton macrostachyus* is a potent source of analgesic and anti-inflammatory principles.

PIF13- EFFECT OF GENDER ON THE ANGIOGENESIS AND INFLAMMATORY PARAMETERS IN THE RAT AIR POUCH MODEL OF INFLAMMATION

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Introduction: Air pouch is a well-established inflammatory model in which fluid extravasations; leukocyte migration, angiogenesis and other parameters involved in the inflammatory response can be measured. In this study the influence of gender on inflammatory parameters has been examined. **Methods:** To induce air pouches, wistar rats were anesthetized. 20 ml and 10 ml of sterile air were injected subcutaneously on the back on day 0 and day 3 respectively. On day 6, inflammation was induced by injection of 1 ml of carrageenan 1% into pouches. After 6h and 72h, the rats were sacrificed and pouch fluid was collected in order to determine exudates volume and cells were counted using cell counter. Pouches were dissected out and the weight determined. Angiogenesis of granulomatous tissue was assayed using hemoglobin kit. **Results:** Six hours after carrageenan injection, the volume of exudates in female rats was significantly ($P<0.01$) greater than male rats but the number of leukocytes and granulomatous tissue weight in female rats were less than male rats. The volume of fluid, the granulation tissue weight and the levels of hemoglobin in the granulation tissue of female rat were significantly ($P<0.05$, $P<0.05$ and $P<0.001$ respectively) less than those in male rat whereas the number of leukocytes in female rats was significantly ($P<0.05$) greater than male rats 72h after carrageenan injection. **Conclusion:** The degree of inflammation and angiogenesis induced in the rat air pouch model is gender-dependent, suggesting that gender may be key consideration in the design of inflammation experiments.

PIF14- POTENTIALITIES IN ANTIINFLAMMATORY THERAPY IN THE ALGAE *Galaxaura rugosa* AND *Dichotomaria obtusata*

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Algae are photosynthetic organisms essential nature and with great potential pharmacological. We assessed antiinflammatory and analgesic properties of the macroalgae *Galaxaura rugosa* and *Dichotomaria obtusata* collected in the Jaimanitas Beach on the northern coast of Havana. The extract was prepared in a ratio of 100g

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of fresh seaweed in each 500 mL of distilled water. The homogenate was centrifuged, the supernatant lyophilized. In the evaluation of antiinflammatory effect of the aqueous extracts of both algae on the ear edema induced by PMA (tumor promoter 13 acetate-12 miristato forbol) in male mice OF1, showed that all doses tested (12.5, 25 and 50 mg / kg) were able to reduce the formation of edema of the ear, when administered intraperitoneally (ip). The aqueous extracts of both algae showed great analgesic activity through the test of nociceptive contortions induced by acetic acid (0.8%) at all doses tested (100, 200, 400 and 800 mg / kg) when administered orally, and (12.5, 25, 50 and 100 mg / kg) administered by the ip. The anti-inflammatory and analgesic effects of seaweed *G.rugosa* and *D.obtusata* are opening new prospects for the possibility of therapeutic use of these algae as widely distributed in our archipelago.

PIF15- IL-1β FURTHER ATTENUATES DIMINISHED ANALGESIC EFFECT OF MORPHINE IN DIABETIC MICE

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It is known that diabetic mice are less sensitive to the analgesic effect of morphine. Some factor(s) derived from mononuclear cells, e.g. interleukin-1β (IL-1β), may be responsible for the diminished analgesic effect of morphine in diabetic mice. Therefore, we aimed to examine direct effects of IL-1beta, intracerebroventricularly (i.c.v.), on morphine-induced analgesia, subcutaneously (s.c.), in diabetic and control mice by using the tail-flick test. Morphine at doses of 1, 2 and 5 mg/kg (s.c.) produced dose-dependent analgesia in diabetic and control mice but diabetic mice were less sensitive to the analgesic effect of morphine when compared to the controls. IL-1β at a dose of 0.1 ng/mouse produced analgesia in control mice but not in diabetics, whereas IL-1beta at a dose of 10 ng/mouse produced a hyperalgesic effect both in diabetic and control mice. IL-1β at a dose of 1 ng/mouse has neither an analgesic nor a hyperalgesic effect in control and diabetic mice. Administration of a neutral (neither analgesic nor hyperalgesic) dose of IL-1β, 1 ng/mouse (i.c.v.), just prior to administration of morphine (s.c.) abolished the analgesic effect of morphine at doses of 1, 2 and 5 mg/kg in control mice and the analgesic effect of morphine became similar to that in diabetics. The diminished analgesic effect of morphine in diabetes was attenuated further with IL-1β at a dose of 1 ng/mouse (i.c.v.). These results suggest that the decreased analgesic effect of morphine in diabetes may be related to IL-1β.

PIF16- EFFECTS OF *Cynodon dactylon* (L.) pers. ON CARDIAC HEMODYNAMIC FUNCTIONS DURING ISCHEMIA AND REPERFUSION

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Introduction: *Cynodon dactylon (C. dactylon)* is widely used in folk medicine of different countries. In the present study, effects of *C. dactylon* on cardiac hemodynamic functions during ischemia and reperfusion (I/R) were investigated in isolated rat heart. **Materials and Methods:** The isolated hearts were divided into four groups (n=7 in each group) then subjected to 30min regional ischemia followed by 120min reperfusion and perfused with rhizome hydroalcoholic extract of *C. dactylon* (50, 100 and 200µg/ml) throughout I/R with a perfusion pressure of 100 cmH_2O. **Results:** During ischemia, left ventricular developed pressure (LVDP) and rate pressure product (RPP) were significantly increased in the group received 50µg/ml of the extract ($p<0.05$) compared to the control. However, the effect was not significant by higher concentrations. Coronary flow rate

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(CFR) was not significantly changed throughout the ischemia. At the reperfusion phase, *C. dactylon* (50µg/ml) elevated LVDP phase ($p<0.05$) but the higher concentrations failed to increase the parameter. Compared to the control value, RPP was not changed by *C. dactylon* while perfusion of the hearts by 50µg/ml of *C. dactylon* produced significant improvement in RPP versus other concentrations. At the same time, CFR was lowered by all concentrations of *C. dactylon.* In this study, we also showed that perfusion of the extract produced a marked and concentration-dependent positive inotropic effect. **Conclusions:** The results indicated that *C. dactylon* produced protective effects during I/R in isolated rat hearts probably by increasing the myocardial contractility and improvement of hemodynamic factors.

PIF17- EFFECT OF PERINEURAL BETAMETHASONE ON NERVE COMPRESSION DURING SPINAL COLUMN SURGERY

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Introduction: Steroids has shown be useful in the treatment of radicular pain, due to its potents properties as anti-inflammatory and neural membranes stabilizadors drugs on spinal and supraspinal sities, despite the fact that the short and long-term results of such therapy remain controversial because the high incidence of side effects. The purpose was to determinate the effectiveness and safeness of perineural selective nerve-root injections during spinal column surgery with betamethasone on pain associated to the radicular compression in Amalia Simoni Hospital, since January to September 2007. **Material and methods**: Sixty two patients who were referred to perform a spinal column surgery because of lumbar and sacrum radicular pain caused by nerve root compression because slipped disk hernia or channel stenosis, were included and evaluated radiographically and clinically. The patients were assigned randomly in to two groups, the experimental group received 12 mg of betamethasone during the operative intervention and in cases that were highly movilizated or the root was much worked the steroid was used endovenous during 3 days. The control group used the conventional operative intervention, the root wasn't treated. **Results**: In the experimental group the pain was relieved in 85,7%, and in the other group was in 43,1%, and the neurological injuries persisted until 3 month after the intervention. The difference in the pain reduction between the 2 groups was highly significant ($p<0.003$), the side effect incidents was minimal (3,5%). **Conclusions**: The use of perineural betamethasone during spinal column surgery is significantly more effective than the conventional intervention in those cases and is safe.

PIF18- ANTIINFLAMMATORY EFFECTS OF MULTIPLE DOSES OF A DICLOFENAC-LYSINE CLONIXINATE COMBINATION IN RATS

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We have previously demonstrated that diclofenac (DCF) plus lysine clonixinate (CL) combination (CLD) produces an analgesic synergism in experimental inflammatory and visceral pain models. In this work we aimed to evaluate the antiinflammatory properties of a repeated dose scheme of CLD (1:5 fixed proportion) in rats. Carragenin-induced edema was produced in animals and then treated with CLD 4.2 mg/kg i.p. (t.i.d. each/8 h); DCF 1 or 10 mg/kg i.p. (t.i.d. each/8 h) or saline control during the 48 h following the inflammatory stimulus.

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None of treatments showed efficacy before 8 h. Low dose of DCF only reduced inflammation by 7.2 % at the end of the study. In contrast CLD combination and higher dose of DCF produced a decrease of 32 and 49% respectively after 8 h and up to 48 h. Our results demonstrated that CL-DCF combination at reduced dose (3.5 mg/kg CL plus 0.71 mg/kg DCF) is at least as effective as higher dose of DCF alone but with a gastrointestinal adverse effects profile more positive. In conclusion, the CLD combination administered in a repeated dose scheme may be useful in the therapy of inflammatory diseases associated with pain.

PIF19- PHARMOCOKINETICS OF DICLOFENAC-LYSINE CLONIXINATE COMBINATION IN HEALTHY VOLUNTEERS

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Main reason to develop analgesic combinations is to keep or increase the efficacy of the individual drugs while using lower doses. During the development of new drugs is basic to profile the pharmacokinetic properties. The aim of this study was to evaluate the individual pharmacokinetics of lysine clonixinate (CL) and diclofenac (DCF) contained in a new formulation (DCL tablets, 250 + 50 mg, respectively) as compared to intramuscular formulations, Firac® (CL, 100 mg I.M) and a Voltaren®, sodium diclofenac, 75 mg IM, in twelve healthy volunteers in three-period design. Blood samples were obtained on selected times by twelve hours, and plasma level of CL and DCF were measured by liquid chromatography. Pharmacokinetics parameters Cmax, Tmax, T1/2, Vd/F, Cl/F, ABC_{0-t} and ABC_{0-t} corrected by dose (ABC_{0-t}./D) were estimated by no compartmental analysis. CL and DCF in the DCL combination showed faster peak levels than the individual formulations. ABC_{0-t}./D of CL in the combination was not modified but ABC_{0-t}./D of DCF was reduced in the combination (F = 85%), in contrast volume of distribution of DCF in DCL was significantly increased, what may explain the prolonged analgesic effect of combination previously seen in preclinical assays. In summary, these results suggests that the oral newly developed DCL combination shows pharmacokinetic properties probably related to faster and more perdurable analgesic effect as compared to individual drugs.

PIF20- HANDLING OF PAIN IN PATIENTS WITH TERMINAL CANCER, TREATED WITH MORPHINE

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A prospective study was made with the objective of establishing a strategy for managing the pain in patients with terminal cancer, who received treatment with Morphine. This research was made during since January, 2005 to January 2007 in sixty (60) patients registered in the Main Drugstore of Jiguaní Municipality in Granma City. The strategy was designed starting from a bibliographical research about the Cancer pain, its measuring and treatment and the consult with experts, being established the following steps: I) Patient's characterization, II) Evaluation of Prescription, II) Detection, evaluation and treatment of adverse reactions, III) Measurement of pain through the use of an instrument, IV) Introduction of the treatment with pharmaco-therapeutic continuation, and V) Sanitary Education.

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As a result it was obtained that the 50% of the prescriptions were adequate and 198 adverse reactions were identified. The measurement of pain determined the intervention of the pharmacist in the 100 % of the cases because the patients qualified the relieve of pain, the intensity and the humour over the 2(two) points which are values beyond the established limits. The pharmaco-therapeutic continuation allowed 100% of the patients to show a hardly perceptible pain, with partial relieve and good temper on the fourth week. The strategy proposed caused a high impact because it reached 100% of satisfaction in patients and/or relatives treated with appropriate pharmaceutical interventions, which were accepted by the 93.3 % of doctors and the 86.6 % of patients, and favored the patients in a very important way.

PIF21- ANALGESIC PHARMACOTHERAPY ON CANCER PATIENTS ON TERMINAL STAGE

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The pain remains in direct proportion of the development of cancer disease and is become bigger in advances stages. This problem is the main causes that affect the quality of life of the oncology patient. The analgesic drugs play an important role in this problems cause release the pain avoiding the traumatic death painful for the family and the patient. This type of therapy should be used correctly for testing it's effectiveness in the majority of the cases. With the objective of evaluate the quality of the prescription, compliment level and reactions adverse an investigation prospective, descriptive of Indication-Prescription type with therapeutic scheme elements has been done on 32 patients with cancer in terminal stage that were provided by the Santiago de Cuba Main Pharmacy and 21 doctors. As the result the inadequate prescriptions were (91%) and the mistakes of the (43.4%), on the other hand the risky combinations [*Morphine-Amicodex* (17.4%), *Morphine-Furosemide* (5.17%)]. The 62% of patients didn't make the treatment just for avoiding addiction. Adverse reactions appears such as, vomits, nauseas, constipation associated to the Morphine (78.2%), the adverse reactions of clinical moderated signification (49%), lower (51%), and possible imputability (71%). The 100% of the doctors shows that they do not know a good knowledge about the use of the analgesic pharmacotherapy in patients affected by cancer, this aspect affect the quality of the prescription and as a consequence the correct use of this important therapy.

PIF22- APPLICATION OF THE MAGNETIC INDUCTIVE STABILIZER (EIMA®) IN THE TREATMENT OF PATIENTS WITH RHEUMATOID ARTHRITIS

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Introduction: The rheumatoid arthritis (RA) is an inflammatory autoimmune illness, is multifactorial, including immunologic variables, neuroendocrines and psychological, being the pain its main symptom. The electromagnetic therapies are effective for the reestablishment of the electromagnetic field of the body and then recover the state of health. The objective is to evaluate the effect of the therapy with EIMA® in patients with rheumatoid arthritis. **Material and methods**: It was carried out a descriptive, retrospective study, in 30 patients assisted in the Center of Metabolic Therapies of Banderilla, Veracruz, Mexico; in the period of January to July of 2007. An inductive electromagnetic field was applied during 21 sessions without another concomitant treatment.

Results: The biggest number of patients included in the study corresponded to the feminine sex (25 patients) representing 83, 33 % of the total of the sample, prevailing the group of 46-60 year-old age. Among the most frequent symptoms it stood out the pain and inflammation in the articulations in 19 and 6 patients respectively, as well as the rigidity and deformity of articulations, both in 5 patients. 100 symptoms were reported at the beginning of the study to diminish up to 31 after the treatments with EIMA®. About 83, 3 % of the total of patients was reported as improved, 16, 7 % continued equally and none increased during this period. **Conclusions**: The treatment with EIMA® was useful to achieve the reduction of the symptoms of patients affected with RA, diminishing significantly starting from the 10th treatment session.

PIF23- EFFECT OF THE TREATMENT WITH MONTELUKAST IN PATIENTS ASTHMATICS

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Introduction: The asthma is defined as a chronic inflammatory disorder of the air roads, in which they participate many cells and cellular elements being demonstrated that the leukotrienes is involved in the rough constriction of the bronchial asthma. Using you as therapy, the calls Antileukotrienes that have the antagonise function before the inflammatory actions mentioned, among them the Montelukast acts impeding the liberation of the processes that take place in the asthma. **Material and Methods**: I take a sample of 100 patients (50 children and 50 adults) asthmatic persistent moderate - severe with understood ages of 6 to 16 years the paediatrics ones and of 17 at 65 the adults of the Paediatric Hospitals of the Hill and The Hospital Salvador Allende for a period of 6 months with studies haematological before the beginning and later to the same one (creatinine, transaminases, complete hemograma) they were also carried out before breathing functional tests and after the treatment. I administer you a dose of 5mg of Leukast (Montelukast) once a day for children of 6 to 14 years and starting from that age 10mg. **Results**: Clinically we obtained a clinical improvement referred by the total of the patients' treaties, being confirmed with the breathing tests that 90% improved its lung capacity and the rest 10% doesn't worsen its parameters in this period. Any patient presents renal, hepatic alterations neither haematological being verified by the evolutionary laboratory studies. **Conclusions**: From the social point of view to these patients and their relatives that have limited lifestyles are able to improve their quality of life with the use of this medication. From the economic point of view he/she diminishes the medication cost administered in the crises and for lost of labour days and for concepts of hospital revenues.

PIF24- TREATMENT OF ATOPIC DERMATITIS WITH AN AQUEOUS EXTRACT OF *Mangifera indica* L. (VIMANG®)

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Introduction: Mango stem bark has been traditionally used in many countries for the treatment of menorrhagia, diarrhoea, syphilis, diabetes, scabies, cutaneous infections, anaemia, etc. Using an aqueous extract obtained by decoction as reported in the *Napralert Database.* A cases study was carried out in two patients with atopic dermatitis treated with a new natural product named *Vimang*®, an aqueous extract of *Mangifera indica* L. stem

bark, which has been registered as antioxidant and anti-inflammatory. Material and methods:18 and 26 years-old females with major and minor clinic criteria and histological diagnosis of atopic dermatitis, were involved in the study and treated topically with *Vimang*® cream (1. 2%), two times a day during six weeks. Total serum immunoglobulin E was determinated for recruitment. Dermatitis Assay Score Index (DASI) was the gold point for evaluation of patients, it was calculated every week. Results: A decrease in DASI value was observed for both patients, -96,6 % and -100 % respectively at the end of the treatment. No adverse events were founded. Conclusions: These results open the way to further clinical researches about the use of Vimang® as an alternative for the treatment of cronic dermatitis like the atopic form.

PIF25- OXIDATIVE STRESS AND ADVERSE OUTCOMES IN PATIENTS WITH CORONARY ARTERY DISEASE

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Introduction: Atherosclerosis is a chronic inflammatory disease and atherosclerotic plaques are result of continuum molecular and cellular interaction between vessel wall and blood constituents, release of number of signaling pathways and activation of the inflammatory and coagulation cascades that lead to structural changes. In some people it has rapid progress and triggers a vicious circle that leads to clinical manifestations of coronary artery diseases, cerebrovascular diseases, and peripheral arterial diseases. Current understanding of molecular basis of atherosclerosis has shown that oxidative stress, oxidative damage to the endothelial cells represents a base of inflammation and development of atherosclerosis. Addressing the oxi-LDL, as a very active and have toxic effect on endothelium, can slow the atherosclerotic progression. **Objective**: The aim of our study was to correlate the level of markers of inflammation oxi-LDL, C-reactive protein, and IL-1, and the onset of chest pain in patients with coronary artery disease. **Materials and Methods:** In a prospective two-centers study, a total of 60 patients with coronary artery disease were included. The plasma samples were obtained at the day of onset of cardiac events (angina pectoris/myocardial infarction) and 4 weeks later, at the day of control visit, free of any cardiac events. The oxi-LDL, IL-1 and CRP were measured by ELISA technique and lipid profile by the biochemical analyzer. **Results:** Our results showed that in target group, the level of oxi-LDL, IL-1 and CRP were significantly higher at the day of onset of cardiac events vs cardiac events free period (p<0.001). **Conclusion**: We conclude that the markers of inflammation oxi-LDL, C-reactive protein, and IL-1 highly correlate with severity of coronary artery disease and with onset of the adverse cardiac events.

PIF26- SYSTEMIC INFLAMMATORY RESPONSE: PHYSIOPATHOLOGY AND MEDIATORS

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Introduction: Inflammatory response characteristics and significance are reviewed. The programmed reactions which are displayed when the reaction is widespread and those mechanisms involved in its onset and ending are analysed. **Methods:** Systematic review of Medline`s bibliography. **Review`s summary:** When injury ensues, there are several programmed reactions displayed which local control is overwhelmed, a quick and generalized reaction promoted by the joint activation of phagocytes and endothelial cells and under humoral and cellular control (complement, citokynes, clotting and fibrinolysis) takes place. As long as this inflammatory reaction is not

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adequately suppressed, a systemic inflammatory syndrome ensues which may disturb the intermediate metabolism and several organ function. Inflammatory mediators are described, as well as their role in the neusoendocrine and in the acute phase reaction. Mechanisms involved in the onset of the inflammatory reaction and its up or downregulation are reviewd. To sum up, we stress the need of systematic approaches to study the different reactions to inflammation if we try to achieve improvements in prognosis and therapy.

PIF27- ELECTRONIC BOOK: INFLAMMATION AND PAIN. ANSWER OF INMUNULOGYCAL SYSTEM AND NEW THERAPEUTIC STRATEGIES

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The inflammation involves many mechanism, some of them induced by citocines released by the fagocytes as answer to the infection by pathogens during the inborn or not adaptive. This can be produced by inflammation and histic damage like consequence of adaptive answer in front to the allergens, taking into evident the participation about the inmunologycal system in this phenomenon so frequent. Keeping in mind the difficulties shown by the medical student about this topic, we made the electronic book using by GIF ANIMATOR and Dreamweaver and created some overrelated pages (chapters) with a principal page (initial page). To it we performed a broad bibliographical revision with the aim to carry out today's elements to those interested people. This electronic book involves elements of interest about the inflammation and the pain, in both entities, so close related make-up a consultation so frequent in all health sense. This will be a useful tool to students of all year of the medical specialities as well as the health stuff people. It make allusion to the physiotherapy of the inflammation of citocin in the genesis of the last one topic as well as the new therapeutic strategies to hold out such disease.

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1st Symposium about Pharmacology of Cytochrome P450 (C)

Chairs: Pedro Camilo Rodriguez, Eva Marrero, Gilberto Pardo, Grisel del Toro, Maria Acelia Marrero, Beatriz Garrido (Cuba)

PC01- GLYCOPROTEIN P GENETIC POLYMORPHISM IN A REPRESENTATIVE SAMPLE OF CUBAN POPULATION

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The human multidrug resistance gene (MDR1) encodes for P-glycoprotein (P-gp) which is a transmembrane transporter protein that conferring resistance to a number of natural cytotoxic drugs and potentially toxic xenobiotics. It plays a protective role for cells against DNA damage. Single-nucleotide polymorphisms (SNPs) in MDR1 gene are associated with phenotypic variation in Pgp expression levels of tissue. SNPs may alter the physiological protective role of Pgp and, therefore, influence disease risk.The wobble C3435T polymorphism at exon 26 has been associated with different expression levels and activity. Differences in allele frequency of the C3435T polymorphism have been demonstrated between distinct ethnic groups. Frequencies of the variant C3435T for p-glycoprotein were evaluated in 140 Cuban unrelated healthy volunteers (73 males and 67 females). Genotyping was performed on peripheral leukocytes DNA by PCR-RFLP method. Genotype frequencies were in Hardy–Weinberg equilibrium. Results showed: 65 subjects (46,4%) expressed CC, 58 (41,4%) expressed CT variant and 17 (12,1%) presented the TT variant. The frequency for C3435T variant of MDR1 gene in Cubans healthy volunteers was similar to Latin America population. Results fit to Cuban population origins.

PC02- DEBRISOQUINE POLYMORPHISM IN A SAMPLE OF CUBAN POPULATION

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There is a large interindividual variability in the extent of metabolism of drugs. Such variability has clinical relevance for widely used medications, for drugs of narrow therapeutic range, if the enzyme pathway plays a major role in the elimination of the drug, or if there are a limited number of therapeutic alternatives; therefore, pharmacogenetic information is an essential for individualized therapy and contribute to a rational use of medications. As the cytochrome P4502D6 (CYP2D6) catalyzes more than 65% of common used drugs, the aim of this paper was to identify different CYP2D6 metabolizer phenotypes in a sample of 104 subjects of Cuban population by determining the metabolic reason between urinary concentrations of debrisoquine and its metabolite 4OH-debrisoquine, quantified by high performance liquid chromatography. We found 3.84% of poor metabolizers of debrisoquine in the studied individuals.

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PC03- INHIBITION OF HUMAN P450 ENZYMES BY NATURAL EXTRACTS USED IN TRADITIONAL MEDICINE

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Different medicinal plants are widely used in Cuba and Mexico to treat several disorders. This work reports *in vitro* inhibitory effects on the P450 system of herbal products commonly used by these peoples in traditional medicine for decades. Experiments were conducted in human liver microsomes. The catalytic activities of CYP1A1/2, 2D6, and 3A4 were measured using specific probe substrates. The *Heliopsis longipes* extract exhibited a concentration-dependent inhibition of the three enzymes, and similar effects were produced by affinin (an alkamide isolated from the *H. longipes* extract) and two synthetic alkamides. *Mangifera indica* L. and *Thalassia testudinum* extracts, two natural polyphenol-rich extracts, diminished CYP1A1/2 and 3A4 activities, but not the CYP2D6 activity. These results suggest that these herbs inhibit the major human P450 enzymes involved in drug metabolism and could induce potential herbal-drug interactions.

PC04- INTERACTIONS ON CYTOCHROME P-450 DRUGS METABOLISM, IN HOSPITALIZED PATIENTS WITH NEUROLOGICAL DISEASES

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Drug interactions involving the Cytochrome P-450 system are common, and generally result from either enzyme inhibition or induction. Hospitalized and poly-medicated patients are more exposed to the drug interactions. It was performed a descriptive and prospective study to determine the incidence of drug interactions, with risky implications for the drugs metabolism in the Cytochrome P-450, given to patients with cerebrovascular disease and other neurological disorders, at General Hospital "Dr. Juan Bruno Zayas " of Santiago de Cuba. The study sample was 531 patients. Risky drug interactions were identified in prescriptions, with an emphasis on those with modification of the drugs metabolism in the Cytochrome P-450. Real and potential interactions were detected, and the drugs more interacting were identified. Patients with ischemic cerebrovascular disease, were predominant in the sample (73.8%). 1802 prescriptions were analyzed, and 57 types of risky drug interactions were detected in 313 patients (59%), of which 38.6% were interactions on the metabolism of drugs in 108 patients (35%). Of the total interactions detected, 77.8% were considered potential, because they were no clinical evidence in patients, 22.2% were real, which were related to failures pharmacotherapy by enzyme induction. Phenytoin and Carbamazepine, were the drugs more interacting, as enzyme inducers with a 79.6% and 42.6% respectively, and Cimetadine as enzyme inhibitor (24.1%). It demonstrates the need to implement health education activities with health professionals related to these interactions so common in the prescriptions, to optimize the pharmacotherapy given to the patients with neurological disorders, third cause of death in Cuba.

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PC05- RESPONSE TO ANTIDEPRESSIVE DRUGS DURING ANTIVIRAL THERAPY IN CUBAN PATIENTS WITH CHRONIC HEPATITIS C

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Introduction: Chronic Hepatitis C (CHC) has worldwide spread out as a pandemic disease, thus affecting about the 3% of the human population. In spite of current available antiviral treatments do not ensure high rates of sustained virology response (SVR), they are related to numerous side-effects and depression one of the most reported ones. **Methods:** A sample of 123 patients having serologic and histological CHC diagnosis were treated with recombinant interferon alpha 2b (3 millions units IM) plus ribavirin (1000-1200mg) during 48 weeks. **Results:** It was obtained a 32.9% SVR, significantly influenced by the side-effect induced lack of adherence to treatment. The 28.4% of patients reported depressive events and were treated with tricycle anti-depressives metabolized by CYP 2C9, 2C19 y 2D6 cytochromes. Observed variability in the response to tricyclic anti-depressives could be related to the genetic and phenotypic variability of the sample as it have described for other populations. **Conclusions:** There are necessary to study the cytochromes polymorphism in untreated, under treatment and 6 months post-treatment CHC patients for knowing if this factor is one determinate on the response of the Cuban patients to anti-depressive treatment.

PC06- TENDENCIES IN THE INVESTIGATIONS AND PUBLICATIONS IN HERBAL-DRUG INTERACTIONS

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Introduction: There is a lack of basic knowledge about the affirmation that herbs, vitamins, and other dietary supplements may augment or antagonize the actions of prescription and nonprescription drugs. This event should be taking account as to the indications for use and safety of herbal medicines regarding the possibility of herbal-drug interactions. **Objectives:** To review the literature for evidence and tendencies on the use, safety and pharmacology of herbal-drug interactions. **Methods:** It was searched the PubMed electronic database papers regarding to “herbal-drug interactions” until December 2007 and compiled data according to the grade of evidence found. **Results:** It was compiled a total of 413 papers related with the approached topic. The drugs more studied were warfarin (3.6%), anti-neoplastic agents (2.0%) and digoxin (2%) and the herbals were hypericum (17.9%), garlic (3.6%), ginkgo (8.2%), panax (6.8%), and kava (4.8%). The major investigations were executed on *in vitro* studies, principally on cytochrome P450 (CYP1A2, 2B1, 2D6, 2E1, and 3A) and P-glycoprotein. On the *in vivo* investigation; it was researched that 21.3% of the papers used animals (rats, 8.0%; mice 3.9%; rabbits 0.7% and others). The countries with more publications were USA (33.9%), Canada (5.8%), United Kingdom (5.3%), Japan (4.1%), Germany (3.9), Australia (3.4%), and Italy (2.4%); the principal authors and Institutions were also from these countries. There was an increase in the published articles from 1999 reaching the maximal production in 2006 (74 papers). The journal with the major number of papers was Br J Clin Pharmacol (13). The type of article more written was the Review (24.9%) and the English was the language used in these papers (91%). **Conclusions:** From 1999 there is an obvious interest regarding the herbal-drug interactions manifested in the increasing of the number of paper published about this topic. These investigations are not sufficient if we taking account the herbal medicine is one of most popular choices of complementary therapies and it could induce interactions with the conventional medicines translated in a deficient treatment or adverse reactions that conduce to an erroneous therapy.

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1st Internacional Workshop of NeuroImmunology (NI)

Chairs: Jean A Merrill (USA), Nancy Pavón (Cuba)

PNI01- BONE MARROW CELLS TRANSPLANT ON THE MOTORS ASYMMETRIES AND THE MEMORY-LEARNING DYSFUNCTIONS IN RATS WITH STRIATAL LESION WITH QUINOLINIC ACID

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Huntington Disease (HD) it is a degenerative dysfunction of hereditary with genetic inherited origen . An effective treatment doesn't exist, the illness advances inexorably to finish in total inability or death after 15 or 20 years. Numerous studies have been carried out for finding an effective treatment for the clinical manifestations that appear in these patients, many of which include medical treatments and more recently the use of cells transplant of different sources: neural and non neural, example of this last one includes to the bone marrow cells (BMC). At present time it is known that it is possible to reproduce, the characteristics of the illness in experimental models [E.g. the pattern of striatum lesion for injection of quinolinic acid (QA)] to be able to look for possible therapies. The restorative effect of the BMC is not known in this experimental model. For these reason the hypothesis of this work consists on if the BMC transplanted in inside in the experimental models injured with QA are able to survive and to modify the behaviour alterations present in this model of Huntington in rats. They were organized different experimental groups: Group I: Group Healthy Control; Group II: Lesion group with QA only. Group III: Group Control of the lesion for injection of QA (lesion with physiologic saline solution; PSS); Group IV: Group of BMC transplant, and the Group V: control group of transplant (transplants of DMEN without BMC), the used sample was (n=10) for each group. In all the cases it was evaluated the conduct activity and cognitive and they were carried out morphological studies. The results point toward an improvement in the behaviour and memory of those animals treaties with BMC transplant. The result allowed increasing the knowledge on the effect BMC transplant to improve the neurological deficit that one observes in the Huntington model in rat. This will be of great value when evaluating this to proceed as therapeutic method for the suffered patients of HD.

PNI02- BIOCHEMICAL AND BEHAVIORAL EFFECT OF GLUTATHIONE DEPLETION IN RAT BRAIN

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Our purpose was to test the effects of depleting tissue glutathione (GSH) by buthionine sulfoximine (BSO, 10mM, intracerebroventricular (icv)) on brain oxidative metabolism and cognitive performance in rats. The experimental groups were: icv BSO–treated at 24h and 48h, icv saline-treated at 24h and 48h and untreated groups. 24h after BSO treatment, GSH levels in hippocampus dropped down to 55% as compared to untreated animals, 48h later the hippocampal GSH values showed a slight increase, in contrast, had been significantly restored in striatum and frontal cortex. GSH depletion modified superoxide dismutase, catalase, glutathione

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peroxidase activities, malondialdehide, tumor necrosis factor α and interleukine IL1 content and DNA damaged markedly in the BSO-treated group as compared to untreated animals in 24h and 48h and saline-treated group followed 48h, however the way in which it occurs is different for each indicator. Glutathione depletion did not influence the performance of animals in the step-through passive avoidance test in the BSO treatment, but impairs the acquisition in the Morris water maze when given before training, and affect the retention at the following day. BSO-treated group at 48h was significantly better than the performance of the same group at 24h. Our results support that glutathione status is a key piece acting in the regulation of brain function

PNI03- DOPAMINE EFFECTS ON STRIATAL MITOCHONDRIAL FUNCTION

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Dopamine (DA) is able to induce neurotoxic effects in several neurological and psychiatrics diseases. It has been postulated that dopamine may generate changes on mitochondrial function by inhibition of respiratory chain. The aim of this work was to determine the *in vitro* effects of dopamine on striatal mitochondrial function. Dopamine (DA) and oxidation products have been related to mitochondrial dysfunction. Striatal submitochondrial membranes and intact mitochondria and were incubated with different DA concentrations for 5 minutes. Significant changes were observed in state 4 oxygen uptake (resting respiration) after 1 mM dopamine incubation. A 35% decrease in state 3 oxygen uptake (active respiration state) was found after 1 mM dopamine incubation. Incubation of submitochondrial membranes with 0.5-0.75–1 mM dopamine inhibited cytochrome oxidase by 38%, 46% and 53% respectively showing the possible inhibition or mitochondrial respiration through the inhibition of cytochrome oxidase. Complex I activity was significantly inhibited by incubation of striatal submitocohondrial membranes with 1 mM dopamine. Hydrogen peroxide production significantly increased by 91% 1 mM DA incubation. Also, DA was able to induce striatal mitochondrial membrane depolarization.
Our results suggest that in our study conditions, DA modifies striatal mitochondrial function trhough inhibition of oxygen consumption, inhibition of cytochrome oxidase activity, increase in hydrogen peroxide production and mitochondrial membrane depolarization.

PNI04- EFFECTS OF THE MK-801 TREATMENT ON THE AMINOACID NEUROTRANSMITTER RELEASE AND CELL DEATH PROCESS IN PEDUNCULOPONTINE NUCLEUS OF HEMIPARKINSONIAN RATS

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The pedunculopontine nucleus (PPN) is a heterogenous structure and it is reciprocally connected with the basal ganglia. The PPN receive a dopaminergic projection from SNc, a glutamatergic projection from STN and also a gabaergic innervation from output nuclei of basal ganglia. On the other hand, it sends cholinergic and glutamatergic pathways to the SNc and NST. *Objective*: the study of the impact of the glutamatergic manipulations (through the STN lesion and antagonist glutamatergic administration) on the aminoacid neurotransmitter extracelular concentrations in PPN. *Methods*: the neurotransmitter release studies were carried out employing the microdialysis cerebral technique follow up high performance liquid chromatography (HPLC) coupled flourimetric detector. The extracellular concentrations of glutamate (GLU) and γ-aminobutiric acid (GABA) were measured. The following experimental groups were organized: Non Treated rats (n=13), 6-OHDA

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in SNc (n=11), Systemic treatment (before and during the month of the 6-OHDA lesion) with MK-801 (5mg/kg of weight, ip) (n=15). Several others groups were organized as control groups. *Results*. MK-801 treatment induced a significant decrease of the Glu extracellular concentration in PPN and also promoted a less loss of dopaminergic cell bodies in the tegmental ventral area. In addition, this pharmacological procedure avoids the cell death process in PPN ipsilateral to the SNc lesion. *Conclussions:* these results are in agreement with the current model of the basal ganglia functioning. Furthermore it suggests a very important neuroprotective role to the glutamatergic antagonist treatment at least adjusting the imbalance of the neurotransmission associated with the 6-OHDA lesion at the PPN level.

PNI05- TRANSPLANTATION OF STROMAL CELLS SUSPENSIONS IN STRIATUM NUCLEUS IN THE RAT MODEL OF PARKINSON'S DISEASE

Pavón-Fuentes N, Blanco-Lezcano L, Martínez-Martín L, Castillo-Díaz L, Cuétara-Bernal K, García-Miniet R, Lorigados-Pedre L, Coro-Grave de Peralta Y, García A, Macías-González R.

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Introduction. A good deal of evidence currently exists to show that transplanting foetal mesencephalic tissue can produce symptomatic benefits both in patients and in disease models. Nevertheless, the technical and ethical difficulties involved in obtaining enough suitable foetal cerebral tissue have been a serious obstacle to its application. Stromal cells derived from bone marrow, due to their potential capacity to generate different types of cells, could be an ideal source of material for cell restoration in neurodegenerative diseases. Aims. Our aim was to evaluate the effect of transplanting stromal cells derived from bone marrow on the behavior of 6-OHDA rats, when they are inserted into the striatum. **Materials and methods**. In this study we used rats with a lesion in the substantia nigra induced by 6-hydroxydopamine, divided into several experimental groups. Rotary activity induced by D-amphetamine (5 mg/kg, intraperitoneally) was evaluated before and throughout the three months following the transplant in all the experimental groups, except in the group of healthy controls. Hemiparkinsonian rats received a total of 350,000 foetal ventral mesencephalic cells and 8 × 104 stromal cells/μL, which were implanted in the striatum. **Results and conclusions**. Animals with stromal cells transplanted in the body of the striatum significantly reduced the number of turns induced by amphetamine ($p < 0.05$); yet this reduction was not greater than that induced by foetal mesencephalic cell transplants. We were also unable to demonstrate any significant improvement in the motor skills of the forelimbs.

PNI06- EVALUATION OF THE IMMUNOLOGIC CHANGES IN THE TEMPORAL LOBE EPILEPSY BEFORE AND AFTER OF THE LOBECTOMY

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Experimental and clinical data explain the roll of certain immune mechanisms in the pathogenic of the epilepsy. This work carries out an immunologic study in 18 patients with temporal lobe epilepsy (TLE) ressistant to pharmacs, before and after 2 years of surgical treatment (lobectomy standard adjusted by electrocorticography). We quantified in blood the albumin and the immunoglobulins (G, M, A) using radial immunodifussion methods, while in cerebral spinal fluid (CSF) the albumin and the IgG levels were evaluated by the same test. In both fluids (blood and CSF) the lymphocytes markers were quantified: CD3+ (pan T), CD4+ (helper/inductor T lymphocytes), CD8+ (supressor/cytotoxic T lymphocytes), CD20+ (B lymphocytes), CD25+ (IL-2 receptor) and HLA-DR by means of an immunocytochemical technique. At central level the functional state of the blood brain barrier (BBB) and intratecal synthesis of IgG were evaluated using the Reibergrame. The results evidenced a

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significant increase in the CD8+ lymphocytes and the activation markers percentage (CD25 and HLA-DR, p< 0.05), both in the preoperative state. A significant increment was observed (p< 0.05) in the intratecal synthesis of IgG, as well as a tendency to the increment of the permeability of the BBB between the first and 6th months the evolution of the surgery. A normal percentage of CD8+ lymphocytes and the activation markers were detected normal at the first year of surgical. These results evidence the presence of inflammatory processes at central nervous system related with surgical proceeding and a normalization of the immunologic parameters in patients with TLE when the epileptogenic area is dried up.

PNI07- CHARACTERIZATION OF NEURON DAMAGE IN NEONATAL HYPOXIA USING MOLECULAR MARKERS

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Introduction: Neonatal hypoxia is a great problem because of the high morbidity and secondary neurological sequelae produced. The objective of this paper is to characterize the neuronal damage by using three enzymes: neuron-specific enolase (NSE), lactate deshydrogenase (LDH) and aspartate aminotransferase (ASAT) in a group of neonates with hypoxia. Material and methods: Fourteen newborn children with neonatal hypoxia were studied. Blood samples were obtained at 24 and 72 hours after birth from each patient. NSE, LDH and ASAT were quantified in all samples. Results: High NSE mean values were observed at 24 hours with a non significant decrement at 72 hours. High LDH mean values were found at 24 hours and with non significative lower levels at 72 hours. Same situation occurred with ASAT but with a non significative tendency to recover normal values at 72 hours. Conclusions: NSE is the best marker for the sample studied. It could be possible to use this enzymatic triade to characterize neuronal damage during the hypoxia process in newborn babies.

PNI08- PROLONGED SURVIVAL AND MIGRATION OF BONE MARROW-DERIVED STEM CELLS TRANSPLANTED INTO BRAIN LESIONS

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Bone marrow-derived stem cell transplantation is a potentially viable therapeutic option for the treatment of neurodegenerative disease, thus we evaluated the survival of rats' bone marrow mononuclear cells implanted in rats' brain. The cells were extracted from rats' femurs, and marked for monitoring purposes by adenoviral transduction with Green Fluorescent Protein (GFP). Labeled cells were implanted within the area of rats' striatum lesions that were previously induced employing quinolinic acid-based method. The implants were phenotyped by monitoring CD34; CD38; CD45 and CD90 expression. Bone marrow stromal cells were extracted from rats' femurs and cultivated until monolayer bone marrow stromal cells were obtained. The ability of bone marrow stromal cells to express NGF and GDNF was evaluated by RT-PCR. Implanted cells survived for at least one month after transplantation and were dispersed from the area of injection towards corpus callosum and brain cortex. The transplanted cells were expressing CD34; CD38; CD45 and CD90. Interestingly, passaged rat bone marrow stromal cells expressed NGF and GDNF mRNA. In conclusion, the bone marrow cells could be

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successfully transplanted to the brain either for the purpose of trans-differentiation, or for the expression of desired growth factors. Abbreviations: CNS, Central Nervous System; GDNF, Glial-Derived Neurotrophic Factor; GFP, Green Fluorescent Protein; NGF, Nerve Growth Factor; PBS, Phosphate Buffered Saline; RT-PCR, Reverse Transcriptase-Polymerase Chain Reaction.

PNI09- HAPTOBLOBIN/IgG INDEX vs SCORE´S BOYERI TO DIFFERENTIAL DIAGNOSIS OF THE MENINGITIS

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Introduction. The meningitis is one of the most severe diseases in children due to its mortality or sequels. However, timely knowledge of whether the infection is bacterial or viral in origin and applied a specific therapeutic would be beneficial for the patients and clinician. **Patients and methods.** Simultaneous serum and cerebrospinal fluid were obtained from 39 pediatric patients, 14 suffering from viral meningoencephalitis and 25 from bacterial meningoencephalitis. Five control cases were examined too. Haptoglobin, IgG and albumin were quantified in both fluids by radial immunodiffusion. Haptoglobin index and haptoglobin/IgG index were calculated. Boyer's score was applied in order to evaluate its diagnostic accuracy. The clinical relevance of Haptoglobin/IgG index and Boyer's score were compared by the analysis of the Receiver-Operating Characteristic (ROC) curves. **Results**. Haptoglobin index was higher not statistically significant in viral meningoencephalitis in comparison with bacterial disease but both were statistically significant with respect to control group. Increased haptoglobin/IgG index were statistically significant in viral meningoencephalitis in relation with viral bacterial meningoencephalitis. There were no association between Boyer's score and the cause of the meningitis. The haptoglobulina/IgG index presents bigger precision for the differential diagnostic of the bacterial and viral meningitis than the scale of Boyer according to the curves ROC. **Conclusion**. The index haptoglobina/IgG showed its diagnostic superiority with relationship to the scale of Boyer to establish the origin of the meningoencephalitis

PNI10- TOXICOLOGICAL EVALUATION OF A NEW THERAPEUTIC OPTION IN NEUROLOGICAL PROCESSES, USING THE INTRACRANIAL INOCULATION

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Usually the interferons have been defined as proteins that exert an inespecífic antiviral action in homologous cells reason why they have been used in cancer therapy considering that is a powerful tool of natural defense system. The anti-tumor action of Interferons (IFNs) is mediated by the inhibition of the tumor cells growth, by the induction of apoptosis and differentiation of these. These characteristics make them as pleiotropic proteins with diverse functions that can exert anti-tumor action stimulating different functions of the organism necessary to control and/or to inhibit the cellular growth. Based on these premises, it is tried to use a combination of human IFN alpha + IFN recombinant gamma like a new therapeutic strategy in neurological processes of neoplasic etiology. The objective of this work was to demonstrate the security of this combination, for its application to the treatment brain tumors. For that purpose, we designed a study of acute toxicity, using rats Sprague Dawley,

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treated by intracraneal route with doses that reached 4, 6 and 12 times above the therapeutic dose. The results showed that the administration of this combination did not cause metabolic or behaviors alterations that could be translated like adverse effects. The histopathologic evaluation confirmed that the administration of this combination does not induce changes in the cellular morphology of the studied organs. We can conclude that combination IFN alpha + IFN recombinant gamma constitutes a new safe therapeutic alternative in neurological processes.

PNI11- BONE MARROW STROMAL CELLS PRODUCING BDNF AND GDNF

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Introduction: Bone marrow stromal cells (BMSC) have attracted interest through their possible use for cell therapy in neurological diseases. These cells are stem cells from the adult marrow and they can give rise to both mesenchymal and non-mesenchymal lineages. BMSC are multipotent and easily available from aspirates of whole bone marrow. Recent reports have demonstrated that BMSC are able to migrate extensively throughout the adult animal and have potential for neuronal differentiation after transplantation into the brain. *Aims:* The objective of this work was determine whether rat BMSC express BDNF and GDNF, in order to study its potential application for treatment of neurodegenerative diseases. **Materials and Methods:** BMSC were harvested from male rats and cultured in α-MEM supplemented with 10% fetal bovine serum. At passage 2, 7 and 12; total RNA and protein were isolated using TriZol reactive. RT-PCRs to evaluate the expression of BDNF and GDNF mRNA using specific primers were carried out. Expression of protein content was evaluated by Western Blot. GDNF production by individual cells in culture was study by Inmmunocytochemistry. **Results and Conclusions:** Our results indicate that rat BMSC have potential to produce BDNF, at least until passage 12. GDNF production from these cells only takes place in passage 7 and 12; therefore the expression of this neurotrophic factor from BMSC varies with the culture growth stage. The ability of BMSC to produce neurotrophic factors supports the use of these cells as a cellular source in the treatment of neurodegenerative disorders.

PNI12- PHYCOCYANIN EXERTS AN ANTI-INFLAMMATORY EFFECT ON THE CNS IN A MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Introduction: Several new approaches to multiple sclerosis (MS) treatment derive from clinical trials based on positive preclinical indications relying on experimental autoimmune encephalomyelitis (EAE). New clues to the pathogenesis of MS and new potential MS surrogate markers are based on EAE pre-clinical research results critically linked to actual findings in MS. **Materials & Methods:** The present study evaluates the effect of systemic c-Phycocyanin (c-Pc) administration in male Lewis rats where EAE was induced immunizing with rat spinal cord encephalitogen in Freund's complete adjuvant. Particularly, we measured the severity of clinical signs and mortality, redox biochemical biomarkers in brain homogenates and serum, and performed ultrastructural studies of the brain and spinal cord sections using transmission electronic microscopy. **Results:**

c-Pc doses of 25 mg/kg significantly decreased the mean cumulative score of EAE when administered in a prophylactic or therapeutic schedule. Biomarkers of protein and lipid damage were significantly ($p<0.05$) increased in EAE rat serum samples, compared to controls. Importantly, protein and lipid biomarkers serum levels of c-Pc treated animals were close to those of control rats. A significant ($p<0.05$) increase in markers of bio-molecule damage in brain homogenates was detected in EAE, on the other hand prophylactic or therapeutic interventions with c-Pc attenuated the oxidative damage. Ultrastructural analyses of brain and spinal cord in EAE induced rats showed a myelin loosen, wobbly and unfastened, most axons had huge mitochondria inside as well, reflecting axon injury. Treatment with c-Pc abolished the axon damage. **Conclusions:** We found that intervention with c-Pc downgrades EAE clinical symptoms, biochemical changes o histological consequences.

PNI13- NEUROPROTECTIVE EFFECT OF DIETARY ZINC SUPLEMENTATION IN A TRANSGENIC MODEL OF SPINOCEREBELLAR ATAXIA SCA-2

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Introduction: Spinocerebellar ataxia SCA-2, is the dominant autosomic molecular form more frequently in Cuba, especially in Holguín. Immunocytochemical stains of Purkinje cells generate esthetical appealing images and atrophy of the idiodendritic tree in patients with hereditary ataxia has attracted much attention. Have a modify genetic animal (AGM) who carries the SCA-2 gen, is a main tool to localize new therapeutic targets to allow at least for improve the way of live and/or turn aside the course of this illness. Preliminary studies have been proving $Zinc^{2+}$ deficiency in the serum and cerebrospinal fluid of these ataxic patients. **Material and methods**: We established two experimental groups, one fed with normal diet and the other with a normal diet enriched with SO_4Zn. These animals were keeping with adequate conditions of temperature, humidity and light. As much the water as the food were given *ad libitum*. We establish the treatment with dietary Zn^{2+} supplementation during at least a year and evaluated histological aspects mainly in the cerebellum. **Results**: We observed a moderated preservation of the Purkinje layer in the cerebellum, with a high number of synaptic terminals and in the molecular layer most of the stellate and basket cells persist. We discuss the role of the zinc as neuromodulator in the cerebellar neurons. **Conclusions**: This evidence will constitute a scientific novelty to help develop new zinc-based therapeutic strategies in front of the evolution of this disease and therefore to consider the emerging roles of zinc in the central nervous system in this illness.

PNI14- EFFECT OF THE AQUEOUS *Mangifera indica* L. EXTRACT (MIE) ON MOTOR CONTROL OF SPINOCEREBELLAR ATAXIA TYPE 2 (SCA2) TRANSGENIC MICE

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The hereditary ataxias are progressive neurodegenerative disorders for which no specific therapy has been identified. Cuba has 125 families that possess hereditary ataxias and the principal molecular form is spinocerebellar ataxia type 2 (SCA2). A gene responsible for SCA2 has been mapped to human chromosome 12 and the disease causing mutation has been identified as an unstable and expanded $(CAG)_n$ trinucleotide repeat. SCA2 is related to the loss of function in the cerebellum, the brain region that controls movement coordination. Beside, they have been suggested that mitochondrial dysfunction, oxidative stress and neurotoxicity processes play an important role in polyQ-induced cell death. MiE have shown anti-inflammatory,

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analgesic and antioxidant properties. These results have been obtained in both *in vitro* and *in vivo* systems. It has also been demonstrated that the extract protects against neuronal cell death following transient ischemia/reperfusion. Therefore, we investigated the effect of the aqueous *Mangifera indica* L. extract (MiE) on motor behavior parameters of SCA2 transgenic mice. A rotating rod apparatus and patagrama tests were used to measure motor coordination of mice. The animals were weighed every week and motor performance was analyzed monthly. Analysis of transgenic mice revealed significant differences of motor coordination compared with its progenitors –no ataxic animals. Daily oral administration of 10-100 mg/kg of MiE markedly improved motor performance of SCA2 transgenic mice. Weight of animals treated with MiE was statically superior than do not treated SCA2 transgenic mice. Finally, patagrama test corroborated that MiE (10-100 mg/kg) improve the motor coordination of SCA2 transgenic mice. Our findings suggest MiE might be a therapeutic agent candidate useful for improving quality of life of SCA2 patients. Further experimental and clinical studies will be needed to clarify the effect of MiE in SCA2.

PNI15- ADMINISTRATION OF *Mangifera indica* L. STEM BARK EXTRACT AND MANGIFERIN IMPAIRS AVERSIVE MEMORY BUT IMPROVES OBJECT RECOGNITION INDEX IN RATS

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An aqueous stem bark extract of the plant Mangifera indica L (MSBE) has been used in pharmaceutical formulation in Cuba for the treatment of immunopathological disorders under the brand name of Vimang®. MSBE and its major constituent, mangiferin (a C-glucosylxanthone), displays antioxidant, antiallergic, analgesic, anti-inflammatory, or neuroprotective actions. Given the several biological actions of MSBE and mangiferin and its potential as a therapeutic agent, it is important to investigate its possible effects on the central nervous system. In the present study we investigated the effects of systemic administration of MSBE or mangiferin on behavioral outcomes of neurological function in rats. A single ip. injection of MSBE 50 mg/kg mangiferin equivalent or mangiferin (10, 50 and 100 mg/kg body weight) given immediately post training produced long-term memory retention impairment for aversive training and a reduction of freezing times, but increases the object recognition index. The administration of MSBE (50mg/kg) and mangiferin (100 mg/kg) given 6 h post training did not affect such memories. There were no significant differences between groups in latency to start exploration, which indicates that MSBE and mangiferin do not affect locomotion or exploratory behavior. The results indicate that MSBE and mangiferin might induce deficits of emotionally triggered memory but, increases object recognition without affecting locomotion, exploratory behavior, or anxiety.

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PNI16- ANTICONVULSANT EFFECTS OF AQUEOUS AND METHANOLIC EXTRACTS FROM *Phragmanthera capitata* AND *Spathodea campanulata* IN RATS

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Phragmanthera capitata and *Spathodea campanulata* are two of the cameroonian medicinal plants used in the treatment of epilepsy. The present work was undertaken to evaluate the anticonvulsant properties of the aqueous and methanolic extracts of the leaves of *Phragmanthera capitata* and barks of *Spathodea campanulata* administrated through intra peritoneal route at the doses of 150, 300 and 600 mg/kg. The effects of different treatments were evaluated on the latency of seizures, the duration, the number of seizures and the onset of death of seizures induced by subcutaneous administration of pentylenetetrazol (PTZ, 100 mg/kg), picrotoxin (PIC, 7,5 mg/kg) and strychnine sulphate (STN, 7,5 mg/kg) and the intraperitoneal administration of thiosemicarbazide (TSC, 40 mg/kg). *Phragmanthera capitata* and *Spathodea campanulata* significantly increased the latency period in seizures induced by PTZ and PIC. They also significantly reduced in a dose dependant manner the duration of seizures induced by the four convulsant agents. These extracts induced a slight increase of the latency of death in TSC-, PIC- and STN- induced seizures but significantly reduced the number of convulsions in TSC-induced seizures. These results show the anticonvulsant activities of the aqueous and methanol extracts of *Phragmanthera capitata* and *Spathodea campanulata* and thereby justify their use in traditional medicine. The aqueous extracts were the most active. *Spathodea campanulata* prove to be potent than *Phragmanthera capitata*. Phytochemical analysis of these extracts shows the presence of triterpenes that can be responsible of their anticonvulsant effects.

PNI17- ANTIBODIES AGAINST NFLP AND NEUROTROPIC VIRUSES IN MULTIPLE SCLEROSIS

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This study shows the antibody central response against neurotrophic virues (measles- rubella- and varicella zoster, MRZ reaction) and neurofilament light protein subunite (NFL) in CSF and serum of Multiple Sclerosis (MS) patients using antibody Index corrected formulation to the analisys. **Patients and Methods:** 28 MS patients with a representative age distribution and gender ratio were analysed for albumin, IgG, IgA, IgM, oligoclonal IgG MRZ reaction and NFL- AI (NV:AI>=1.5). **Results:** Cuban MS patients showed similar CSF data patterns to those reported from a large reference population from no tropical country. Frequencies of intrathecal measles- (78/78%) and varicella zoster- (59/55%) antibody synthesis correspondingly were found. A 50% lower frequency of intrathecal rubella antibody synthesis was found in Cuban patients (30%, gender ratio of rubella -AI positives m:f =1:6) compared with German patients (60% , m:f =1:1.8). NFL-AI was increased in 11/28 cases, range of AI = 1.6-13.9 (median AI= 2.9). The normal values were AI= 0.7-1.2 (median AI=0.95). A few MS cases investigated showed increased NFL-AI combined with any of the antibody species to neurotrophic virues, frequent in MS. From this preliminary sensitive CSF analysis, this approach do no confirmed the statement of the disease course and CSF NFL autoantibody in MS. Nevertheless more studies most be conduced looking for the real relevance of our finding.

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PNI18- MODELING THE ORIGIN OF THE DIFFUSION WEIGHTED MRI (DWMRI) SIGNAL FROM GRAY MATTER IN MULTIPLE SCLEROSIS

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The mechanisms underlying the progressive course of multiple sclerosis (MS) are not fully understood yet. Diffusion weighted MRI (DWMRI) can provide quantitative estimates of brain damage related to MS. Although MS has been considered a disease affecting the white matter, pathological studies have shown extensive myelin loss, axonal damage and neuronal loss in cortical grey matter. In the present work a biophysical model of water diffusion in gray matter is used for studying the effect that MS has on the DWMRI signal. The model propose a more realistic description of water diffusion by modeling the tissue as composed of five separate compartments with individual properties of diffusion and transverse relaxation. The compartments accounts for nucleus and cytoplasm of neurons and glias as well as the extracellular space. Our study assumes slow exchange between compartments. Cell morphology is taken into account by modeling the neurons as ellipsoids and the glias and nucleus as spheres. For investigating the effect of MS on the model simulations were carried out. For this, the intracellular fraction volume was diminished, resulting in a decreasing of the DWMRI signal along b factor. The next step is the estimation of the parameters and the unobserved variables of this model from actual data which could be helpful in the diagnosis of MS and its differentiation from other neurodegenerative disorders.

PNI19- MODELING THE EFFECT OF DEMYELINATION ASSOCIATED WITH MULTIPLE SCLEROSIS ON EEG AND BOLD SIGNALS

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Demyelination in the central nervous system (CNS) occurs due to a variety of pathophysiological conditions. Perhaps the most notable is demyelination associated with multiple sclerosis (MS). MS is thought to be an autoimmune disease characterized by inflammatory lesions throughout the brain and the spinal cord. In this work, we study the effect of demyelination on EEG and BOLD signals by means of a biophysical model linking theses signals to the underlying neuronal activity. The relationship between EEG and neuronal activity was studied with a neural mass model. We model a brain voxel and its interaction with voxels of the same area (short-range interactions) and other cortical areas (long-range interactions). In our approach short-range interactions can be both excitatory and inhibitory and the strength of connections is assumed to decay with the distance between subpopulations of neurons. On the other hand, long-range interactions are only excitatory and its strengths are estimated from human diffusion weighted magnetic resonance imaging data. We made basic physiological hypotheses based on experimental results concerning how cerebral blood flow (*CBF)*, cerebral metabolic rate for oxygen ($CMRO_2$) and glucose (CMR_{glc}) consumption are related to neuronal activity. Equations obtained from these assumptions were combined with the Balloon model for linking neuronal activity and BOLD. For modeling the effect of demyelination, time constants related to the coupling between voxels and brain areas were reduced. Results show that the EEG spectrum is shifted towards lower frequencies while the amplitude of the BOLD signals diminished.

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PNI20- INTENSIVE PHARMACO-SURVEILLANCE OF IFN α2B IN THE TREATMENT OF MULTIPLE SCLEROSIS

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Introduction: Pharmaco-surveillance is the group of activities and methods developed for the study, identification, and assessment of pharmacological treatments acute and chronic use effects. It is fundamental to develop drugs of new application and recent production. The recombinant human interferon alpha 2b (IFN α2br), an antiviral and immunomodulator drug, elaborated in Cuba, is used in different pathologies, among them the Multiple Sclerosis (MS). For its commercialization it is necessary to know their margin of security. **Objective:** To evaluate the adverse reactions of the IFN α2br in patients with multiple sclerosis. **Method:** 70 clinical data of patients that are included in the national clinical trial phase IV, randomized and blind double were reviewed. From these data adverse reactions, including quantity and type were picked up. They were classified into: light, moderate and serious. It was applied Karch and Lasagna´s algorithm to evaluate the force of causality between drug administration and adverse reaction and classify them in: definitive, probable, possible, conditional and not related. **Results:** 53 patients presented 207 adverse reactions to IFN α2b. The most frequent adverse reactions were: fever 16.81%, migraine 14.15%, chills 10.61%, arthralgia 10.61%, asthenia 9.73% and myalgia 7.96%. These adverse reactions were in its majority collateral effects and they were classified as definitive. 197 had a favourable result. No patient reported antibodies anti - IFN α2b. **Conclusions:** IFN α2br is safe and it could be used in the treatment of MS by intramuscular via.

PNI21- GUIDES FOR THE USE OF HUMAN IMMUNOGLOBULIN (INTACGLOBIN®) IN THE TREATMENT OF MULTIPLE SCLEROSIS

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Introduction: Multiple Sclerosis (MS) is an inflammatory and demyelization disease of the Central Nervous System (CNS) described since the XIX century. It is common in young adults. Is etiology is uncertain; the most widely supported hypothesis is the existence of an autoimmune process with a genetic predisposition. The human Immunoglobulin (Intacglobin ®) has an immune-regulatory activity, so it could help to the treatment of the MS. **Objective:** To make a guide that helps the patient to face more scientifically this pathology. **Method:** In order to make a guide, an extensive search that included, books, magazines, articles and web pages, was carried out. **Results:** It was possible to agglutinate easily and scientifically, taken account the Evidence Based Medicine, a document that traces the therapeutic rule in order to achieve an appropriate diagnostic and treatment of the multiple sclerosis using Intacglobin, which is effective in the relapsing–remitting MS form. **Conclusions:** A guide for the treatment of MS with Intacglobin was elaborated.

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PNI22- NEUROIMMUNOLOGY IN PATIENTS WITH MENINGOENCEPHALITIS CAUSED BY *Neisseria meningitidis* VACCINATED AND NOT VACCINATED WITH VA-MENGOC-BC VACCINE

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The morbidity and mortality due to meningococcal disease in Cuba have been diminished by a massive antimeningococcal (VA-MENGOC-BC) vaccination campaign.. However, after some years, some cases c of meningococcal disease in vaccinated children are reported. Objective. To compare the neuroinmmunological behavior and the clinical characteristics of the illness between vaccinated and not vaccinated patients. Patients and Method: A group of children that suffering from meningoencephalitis due to Neisseria meningitides are studied. Simultaneous serum and cerebrospinal fluid were obtained. The IgG, IgA IgM and albumin were quantified in both fluids by radial immunodiffusion. These results were compared with studies carried out in the period prevacunal, Results: The immunoglobulin intratecal synthesis pattern and the mean values of the local synthesis of immunoglobulin was different between the two groups. The disease was less severe in vaccinated group and a single patient development a meningococcemia diseases. Conclusions: The neuroimmunological response and the clinical characteristics of the vaccinated patients were different respect to the not vaccinated patients. It support the effectiveness of our vaccine at the same time future strategies should be improve in order to prevent of the meningococcal disease in our country.

PNI23- EVALUATION OF THE EFFICACY AND TOLERANCE OF THE NEUROLOGIC RESTORATION PROGRAM (NRP) IN PATIENTS WITH TRAUMATIC MEDULAR LESIONS

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The spinal cord medullar lesions constitute one of the cause that produce most sequels in a young population. We evaluate the efficacy of the applied to a number of patients with spinal cord injuries, with the objective of determining demographic and clinical characteristics of such patients. When the patients are evaluated using quantitative neurologic scale –Keeping in mind the neurological level, severity of the lesion, time of evaluation and duration of the treatment as prognostic factors of the above mentioned program. **Material and methods:** A study was conducted on 50 patients (25 patients and 25 controls) with T5-L2 raquimedullar lesions of the traumatic aetiology that were assisted at CIREN for a year. **Results:** 58% of patients were between 19 and 29 years of age, with predominance of males, most frequent aetiology was the traumatic one, and most frequent neurological level was of D9-L2, 48% of the sample had an evaluation time of 3-5 years. In those patients submitted to treatment a significant improvement was evidenced in Barthel and ASIA scales as well as contractures. Good tolerance to the therapeutic program was thus reported. Time of evolution, severity of lesion as well as duration of the neurological restoration program constitute prognostic factors for the medullar lesioned patient.

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PNI24- MODULATION OF THE EFFECT OF BAD NEWS IN THE ETHIOPATHOGENIC COURSE OF MULTIPLE SCLEROSIS

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Objectives: To value the persons' opinion with Multiple Sclerosis in relation to the communication of bad news and to propose a model to facilitate the modulation of the effect of bad news in the etiopathogenic course of Multiple Sclerosis. **Material and Method:** To value the persons' opinion with MS related to the communication of bad news, an aleatory sample of 50 persons (32 women and 18 men) was chosen in the city of Havana. These persons were assisted at the Neurology Service from "Calixto García" HCQU, who received a survey related to the investigated problem. Starting from the results, a model was proposed for the communication of bad news. **Results:** 72% of the samples requested to know the real truth and 28% preferred the news to be given to the family. With regards to their disposition for treatment, a 78, 0 % feel they are capable of reducing those factors that hinder them to bear their illness and a 22, 0 % feel their lives will under go a change in style when the disease declines. Considering these results, a model for communication of bad news was proposed, valuing age, the patient's psychological balance, evolution of the disease and its emotional impact, net for social support, as well as the policies to be followed in the communication of bad news. **Conclusions:** To adequately communicate persons with MS, their diagnosis, treatment and prognosis this would bring benefits during the course of the disease.