CONFERENCE ABSTRACTS

Sunday, May 19, 2013

BASIC KNA

KNA 1: CARBOHYDRATES AND T CELLS: A SWEET TWOSOME

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Carbohydrates as T cell-activating antigens have been generating significant interest. For many years, carbohydrates were thought of as T-independent antigens, however, more recent research had demonstrated that mono- or oligosaccharides glycosidically-linked to peptides can be recognized by T cells. T cell recognition of these glycopeptides depends on the structure of both peptide and glycan portions of the antigen. Subsequently, it was discovered that natural killer T cells recognized glycolipids when presented by the antigen presenting molecule CD1d. A transformative insight into glycan-recognition by T cells occurred when zwitterionic polysaccharides were discovered to bind to and be presented by MHCII to CD4+ T cells. Based on this latter observation, the role that carbohydrate epitopes generated from glycoconjugate vaccines had in activating helper T cells was explored and it was found that these epitopes are presented to specific carbohydrate recognizing T cells through a unique mechanism. I will review the key interactions between carbohydrate antigens and the adaptive immune system at the molecular, cellular and systems levels exploring the significant biological implications in health and disease.

Monday, May 20, 2013

Symposium I: Basic Topics

Chairs: S. Meri (Finland) and L. M. Wetzler (USA)

KNA-2: COULD ADJUVANTS OVERCOME THE THYMUS-INDEPENDENT OF POLYSACCHARIDES?

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Introduction. Vaccination remains the most cost-effective method for preventing infectious diseases. Key to vaccine design is the development of immunological memory, which is an essential property of the adaptive immune system. Bacterial polysaccharide conjugate vaccines are the gold standard currently used to confer protection of the host by inducing humoral immune responses against T-cell-independent antigens. Adjuvants have been used to improve the immune response to vaccine antigens; however, no mucosal adjuvant has been licensed for human use. **Aim.** Describe our progress in the use of mucosal and parenteral adjuvants to achieve significant immune responses against T-cell independent antigens. **Results.** We demostrated the efficacy of a mucosal adjuvant like AFCo1 (Adjuvant Finlay Cochleate) and different AFPL (Proteoliposomes) to elicit high levels of specific antibodies response, subclass pattern, affinity maturation against capsular polysaccharide Vi of *Salmonella Typhi* or *Neisseria meningitidis* serogroup A after a primary response. Also, we evaluated the antibodies response, B, T cell immunity, and memory responses after a booster dose with plain polysaccharide. Vigorous specific B and memory B cell responses after booster dose were induced. Effector memory and central memory T cell responses were developed. **Conclusion.** The combined formulation of capsular polysaccharides with adjuvants like AFCo1 or AFPL provides an improved immunogenicity, in particular with regard to cellular responses and long-lasting and rapidly expanded cells.

KNA 3: COMPLEMENT ACTIVATING PATTERN RECOGNITION MOLECULES (PRMS) OF THE INNATE IMMUNE DEFENCE AND THEIR POSSIBLE ROLE IN NEISSERIAL INFECTIONS

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The complement cascade is activated through the classical pathway, the lectin pathway and the alternative pathway. The first two are initiated through recognition of ligands by complexes of pattern recognition molecules and zymogens. Activation of the C1 complex of the classical pathway is generally believed to be well understood. The signal for the first step, autoactivation of C1r remains mysterious, but most are happy to quote allosteric changes in C1q. In the pentameric complex the two C1r proenzymes activate each other and then the two C1s's of the tetrameric zymogen complex. C1s activates C4 and then C2 to form the C3 convertase, C4bC2a/b. C1 is activated upon binding to immune complexes or some bacterial products. The lectin pathway is more complex. Six different PRMs are involved in binding to ligands of microorganisms or altered-self: three collectins, mannan-binding lectin, CL-L1, CL-K1, and three ficolins, H-ficolin, L-ficolin and M-ficolin. All these PRMs make use of two serine proteases termed MASP-1 and MASP-2 (since they were discovered as MBL-associated serine

proteases), which are sequentially activated. The six PRMs share an overall similar structure being built of subunits, each composed of three identical polypeptides with a collagenous region and a globular pattern recognition structure. This globular structure for the collectins is a C-type lectin domain recognizing a variety of sugars (thus the term mannose-binding lectin is a misnomer for MBL), while the ficolins have a fibrinogen-like structure recognizing acetyl groups in various molecules and patterns. Evidence for interactions with Neisseria shall be presented.

KNA 4: OPPORTUNITIES AND RISKS OF USING COMPLEMENT REGULATOR BINDING PROTEINS AS VACCINES

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Surprisingly many pathogenic bacteria, including *Neisseria meningitidis*, escape complement killing by expressing surface proteins that bind soluble human complement inhibitors (or regulators, Cregs), notably factor H and/or C4bp. The factor H binding protein (FHBP) is a component of a novel multicomponent vaccine against group B meningococcus (4CMenB). Another component is porin A (PorA) as a subcomponent in outer membrane vesicles (OMV) derived from the New Zealand strain. PorA has earlier been shown to bind C4bp. The importance of factor H binding in immune escape of microbes is emphasised by the fact that FHBPs from a large number of microbes have the same specific binding site on factor H. The benefit of having Creg binding proteins in vaccines is that, not only do the vaccine-induced antibodies recognise the microbes but they also neutralise important virulence factors making the target microbe more susceptible to serum- and phagocyte-mediated killing. The problems are that during evolution the microbes have developed a variety of Creg binding proteins and have the potential of further increasing the variety, which may restrict vaccine efficacy. Paradoxically the proteins exhibit variation, while they simultaneously need to maintain the same functional activity – specific binding of factor H. A theoretical risk in using FHBPs in vaccines is that they could lead to the generation of autoantibodies against factor H. This is because FHBP would bind host factor H and form a complex that could be captured by potentially pre-existing FH-reactive B cells in susceptible individuals. The B cells would become activated by help from T cells that would be specific for the microbial component of the complex. Anti-factor H antibodies are a feature e.g. in an autoimmune form of atypical hemolytic uremic syndrome (aHUS). Particularly individuals having a deletion in CFHR-1-3 genes in the Regulators of Complement Activation (RCA) gene cluster in Chromosome 1q32 (≈ 5% of total population) are at risk to develop anti-factor H-mediated aHUS. aHUS develops because autoantibodies block the binding of the surface interactive C-terminal region of factor H to vascular endothelial cells, platelets and blood cells. This would lead to complement-mediated blood cell and vascular damage. Until now, luckily, no reports on vaccine-induced anti-factor H autoantibody formation in humans or in animal experiments have been reported. Nevertheless, it is recommended that individuals vaccinated with FHBP-containing vaccines be monitored for the potential appearance of anti-factor H antibodies, blood cell damage or for vasculitic complications.

KNA 5: MECHANISM OF THE IMMUNE STIMULATING ACTIVITY OF THE NEISSERIAL MAJOR OUTER MEMBRANE PROTEIN PORB: ROLE OF ANTIGEN PRESENTING CELLS AND SYSTEMS IMMUNOBIOLOGY ANALYSIS

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Our laboratory has spent the past two decades exploring the innate immune effects of the Neisserial porins, especially meningococcal PorB. We have found that it can stimulate innate immune cells via TLR2, that this effect mediates a potent vaccine adjuvant affect and that PorB can induce significant humoral and cellular immune responses to antigens when delivered with PorB. All these affects are, also, wholly dependent on the TLR adaptor molecule MyD88. Further studies, using MyD88 FLOX mice, have shown that both dendritic cells and macrophages mediated PorB/TLR2 stimulation are needed for this adjuvant affect to occur. In addition, TLR2/MyD88 signaling in the B cells is also needed for optimal humoral immune responses induced by PorB but this response is not totally dependent on this axis. We have previously shown that PorB/TLR2 stimulation induces proinflammatory cytokines and NFkB nuclear translocation. New data now examines the role of PorB in antigen uptake and antigen presenting cell migration; PorB enhances both aspects of induction of immune responses, and these phenomena are also MyD88 dependent. Finally, microarray analysis of gene expression induced in mice receiving PorB/Ovalbumin vaccines, as compared to those mice receiving OVA alone, revealed that the kinetics of the response in a number of gene sets is accelerated when PorB was used, with maximal expression after the second vaccination, rather than the third vaccination when OVA alone was used. Moreover, a recruitment of a number of innate inflammatory pathways was induced to a much greater extent when PorB was included in the vaccine preparations. These included a number of micro RNAs whose functions we are currently investigating. In conclusion, PorB is a vigorous and multifaceted vaccine adjuvant that works through a number of unique pathways to induce a potent and robust immune response, as good or better than most known vaccine adjuvants.

THE MENINGITIS RESEARCH FOUNDATION MENINGOCOCCAL GENOME LIBRARY

Lucidarme J[1]*, Hill D[2], Jolley K[2], KaczmarskiEB[3], Parkhill J[4], Green J[5], Tang CM[6], FindlowJ[1], Maiden MCJ[2], BorrowR[1], and Glennie L[7]

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Introduction. The open access Meningitis Research Foundation (MRF) Meningococcal Genome Library contains whole genome sequences of isolates from all 514 culture-confirmed invasive meningococcal disease (IMD) cases in England, Wales and Northern Ireland from the epidemiological year 2010/11. The library is intended to support researchers across all areas of meningococcal research including population genetics, virulence and pathogenicity, vaccine design and determining potential vaccine coverage. It also provides comprehensive population data prior to the possible introduction of the meningococcal group B (MenB) 4CMenB vaccine recently granted European Licensure. **Aims.** To summarise the epidemiology of the constituent isolates with reference to those of previous years. We then focus on the potential strain coverage of 4CMenB and several investigational MenB

vaccines against English, Welsh and Northern Irish IMD isolates, based on MRF genome library data. **Results and conclusions.** The proportion of MenB isolates was lower than in previous years but these still accounted for more than three quarters of the 2010/11 isolates. The predominant MenB clonal complexes (ccs) in order of prevalence were cc41/44, cc269, cc213, cc32 and cc60. Capsular group Y accounted for approximately 15 % of isolates, the majority of which belonged to cc23. Capsular group W accounted for approximately 5% of the isolates and comprised cc11 and cc22 isolates in roughly equal measures. Based on genotypic data, 4CMenB and several investigational MenB vaccines offer great promise for broad protection against MenB in England, Wales and Northern Ireland. Extension of the library to include all isolates from 2011/12 is currently in progress.

Monday, May 20, 2013

Symposium II: Diagnostic and Clinical aspects for Meningitis Diseases

Chairs: E. Kaczmarski (UK) and J. Diez-Dominguez (Spain)

RAPID DIAGNOSTICS FOR MENINGITIS DISEASES

UK

Pending Abstract

BACTERIAL MENINGITIS IN CUBA. CLINICAL AND EPIDEMIOLOGICAL SITUATION

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Meningitis remains one of the most devastating infections affecting young children worldwide. A major risk factor is the lack of immunity to specific pathogens associate with young age. Considerable progress has been made understanding the pathophysiology and immunopathogenesis of this disorder but mechanisms underlying shock and multi-organ failure still remain poorly understood. Regardless of etiology, most patients have similar clinical syndromes. Common symptoms include headache, nausea, vomiting, restlessness, and irritability. However, most of these symptoms are nonspecific. In general, viral infections are much common than bacterial infections. Incidence of main bacterial causative agents was *Streptococcus pneumoniae* $0{,}9/10^5$ population, *Neisseria meningitidis* $0{,}2/10^5$ population, and *Haemophilus influenzae* type b (Hib) $0{,}2/10^5$ population. Highest incidence was mainly clustered in the center of the island. Seasonality during September, January, and March was observed. Declining of bacterial meningitis (BM) incidence may be the result of the rational use of preventive and control measures nationwide. The impact of vaccination against *N. meningitidis* serogroup B and C have been initiated since 1989 (vaccination campaign), and and continued in 1991 by the national vaccination schedule as well as the successful vaccination against Hib carried out since 1999 in children. Therefore, continuous vaccination and high coverage (>99%) achieved in population, may explain not only the decreasing trend of the incidence in targeted population, but the decrease in other age groups probably due to herd immunity induction. At present, *S. pneumoniae* remains the major causative bacteria of BM in Cuba without specific vaccination control. The results of the National Program for Control and Prevention of the Neurological Infectious Syndrome evidenced a reduction of bacterial meningitis incidence.

MENINGOCOCCAL DISEASE. BEYOND THE FIGURES

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Meningococcal infection produces terrible diseases. Epidemiologist, public health workers and decision makers know its severity but tend to forget what is behind each one of the cases. Neisseria meningitidis is carried in the nasopharynx, and may invade the organism entering the blood stream.

They can infect the meninges and then produce a direct neuronal lesion and an inflammatory response which leads to neuronal death and occasionally hydrocephalus. Mortality in these cases is lower than sepsis cases, but neurological sequels are frequent. It is estimated that up to 30% of the subjects recovering from meningococcal meningitis have long-term neurosensorial disorders and social inabilities. Occasionally, N. meningitidis, after entering the blood stream releases endotoxins and activates the inflammation mediators, producing sepsis. Sepsis is a complex disease where endothelial damage, capillary leak, and cardiac dysfunction drive to respiratory distress, petechiae and shock. Endotoxin liberation produces DIC. All these mechanisms, and not the direct invasion of the meningococcus are the responsible for the severity of the disease, and explain the high mortality in spite of very potent antibiotics.

LAB SAFE WORKING WITH MENINGOCOCCI

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Neisseria meningitidis is a recognised cause of serious illness and death. In most countries infections are uncommon, typically occuring as sporadic cases in young children with a second smaller peak in adolescence. In recent years a number of reports have highlighted instances of infection in clinical and laboratory healthcare workers. Many legislatures have a requirement for employers to take all reasonable precautions to ensure the safety of staff. Key to managing the risk from meningococci is understanding their ecology, methods of spread and characteristics of both the organism and the individuals who may be exposed. It is recognised that in some age groups up to 30% people may be carriers of meningococci but that only a tiny proportion of these will become unwell. This is because the majority of circulating strains have low or no pathogenic potential and because individuals build immunity to *Neisseria meningitidis* by natural exposure. Strains which have caused clinical illness carry a much higher risk to carers and those undertaking laboratory testing. A spectrum of methods ranging from physical barriers, working in controlled environment workplaces through to prophylaxis by antibiotics or vaccination are available to mitigate and manage the risk. The presentation will review some scenarios to identify the risks arising from clinical and laboratory exposure and steps that can be considered to facilitate safe working. Appropriate use of biosafety cabinets, validating laboratory examination procedures for safety as well as scientific robustness and engagement with occupational health services will be discussed.

Tuesday, May 21, 2013

Symposium III: Meningococcal Vaccines

Chairs: J. Holst (Norway) and G. Enwere (France)

KNA-6: A LONG AND WINDING ROAD: TOWARDS A VACCINE WITH BROAD STRAIN COVERAGE AGAINST MENINGOCOCCAL SEROGROUP B DISEASE

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Wild-type outer membrane vesicle (wtOMV) vaccines against serogroup B (MenB) meningococcal disease havebeen successfully used since the 1980s to prevent clonal outbreaks of MenB disease. Data from large clinical studies and retrospective statistical analyses give effectiveness estimates of at least 70%, and aconsistent pattern of moderate reactogenicity has been seen with the use of approximately 60 million doses of three different wtOMV vaccines. The key limitation of these wtOMV vaccines is their ability to control only disease caused by specific clonal groups. In New Zealand from 2004 to 2008,the wtOMV vaccine MeNZB was used to control a clonal MenB epidemic, providing a number of new insights regarding international and public-private collaboration, vaccine safety surveillance, vaccine effectiveness estimates and communication to the public.The various historic experiences with wtOMV vaccines will also prove important for the next generation of MenB vaccines, which are designed to give more comprehensive protection against diverse circulating strains. A combination of the conventional wtOMV and antigens identified through reverse vaccinology has resulted in a multi-component vaccine, 4CMenB ("Bexsero®"), whichrecently received regulatory approval in Europe. Strain-coverage estimations are in the range of 70% to 90%, depending on the local epidemiological situation.Following implementation of this vaccine, monitoring shouldfocus on effectiveness data for various circulating strains and potential vaccine effects on carriage and herd immunity.

EARLY DEVELOPMENT OF MENBIOVAX VACCINE WITH POTENTIAL FOR UNIVERSAL PROTECTION AGAINST *NEISSERIA MENINGITIDIS*

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Introduction. Meningococcal vaccines are required that provide broad protection against disease-causing strains of multiple serogroups of *Neisseria meningitidis*. Conjugate vaccines that target the polysaccharide capsule are effective against specific serogroups (but not B serogroup). Outer membrane vesicle (OMV) vaccines have been used successfully in epidemics caused by specific B serogroup strains. It is generally accepted that natural immunity to meningococcal infection is based on heterogeneous antibody responses, including those against protein antigens. Recently there have been significant advances in the development of protein based vaccines with potential effectiveness against the majority, but not all, B serogroup disease-causing strains. **Aims.** In the approach to meningococcal vaccine development described here, the aim is to mimic the normal immune response to *Neisseria meningitidis* infection, so achieving the efficacy required for broad protection but avoiding the safety

issue of using a live organism. MenBioVax is designed to contain a mixture of protein antigens, including the highly immunogenic heat shock proteins (Hsps), derived from stressed bacteria. **Results.** Vaccines have been produced from *N meningitidis* that was grown under stress in fermenters. Several culture conditions were investigated including those of elevated temperature and oxygen limitation. These conditions, together with downstream processing, result in vaccines with increased levels of stress proteins, including Hsps. In mice, these vaccines without adjuvants induced broad antibody responses that are bactericidal against *N meningitidis*. **Conclusion.** MenBioVax based on stress protein antigens has the potential to provide protection not only against serogroup B but also against multiple serogroups of meningococci.

A NEW APPROACH TO PREVENTION AND TREATMENT OF MENINGOCOCCAL DISEASE

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Introduction. Susceptibility of infants and young children to invasive meningococcal disease (IMD) is attributed to their inability to respond to polysaccharide antigens; consequently, significant resources were invested in conjugate vaccines. While devastating when it occurs, IMD is relatively rare, indicating natural immunity is developed to these infections agents. Sources of natural protective antibodies to meningococci need to be considered as an alternative approach to prevention. It is accepted that carriage of *Neisseria lactamica* (NL) and non-groupable *Neisseria meningitidis* induces protection. Carriage of *Neisseria* species is very low during the first months of life; however carriage of *Moraxella catarrhalis* (MC) is common. **Aims.** to assess cross-reactive antigens on NL and MC for potential induction of protection against IMD. **Results.** Both commensals had antigens cross-reactive with the major meningococcal virulence factor, lipooligosaccharide (LOS). We demonstrated *in vitro* that the meningococcal L(3,7,9) and L8 LOS immunotypes (identified in 90% of clinical isolates of the major serogroups) induced the highest pro-inflammatory responses. We found over 60% of *M. catarrhalis* obtained from children bound monoclonal antibodies cross-reactive with L(3,7,9). Commensals expressing the cross-reactive LOS induced neutralising antibodies to meningococcal LOS and absorbed bactericidal and opsonising antibodies against meningococci with corresponding immunotypes. **Conclusions.** If immunity to IMD is naturally acquired by carriage of commensal organisms with cross-reactive LOS epitopes, these need to be considered in development of vaccines targeted against the major virulence factor in IMD and the role of mucosal vaccines assessed.

SUBCAPSULAR MENINGOCOCCAL VACCINE ANTIGENS IN THE COMMON CHILDHOOD COMMENSAL, *NEISSERIA LACTAMICA*

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Introduction. The novel, multicomponent '4CMenB' vaccine against capsular group B meningococci (MenB) has recently received European licensure. It includes a PorA P1.4-containing outer membrane vesicle (OMV), factor H-binding protein (fHbp), *Neisseria* Adhesin A (NadA), and Neisserial Heparin-Binding Antigen (NHBA). PorA is only expressed in meningococci. Many subcapsular antigens, however, are shared with non-pathogenic neisseriae that also colonise the nasopharynx. These

commensals are believed to elicit cross-protective immunity against meningococci, especially in infants and children, whilst occupying a niche that may otherwise accommodate pathogens. **Aims.** To assess the potential impact of 4CMenB against the important childhood commensal, *Neisseria lactamica,* by determining the genetic distribution of the primary recombinant 4CMenB antigens among fifty diverse recent isolates collected in England Wales and Northern Ireland. **Results and conclusion.** All of the isolates lacked genes for fHbp and NadA. They each, however, possessed *nhba* alleles that were closely related to those observed among a comprehensive recent panel of invasive MenB isolates from the same broad geographic region. These findings highlight the potential for 4CMenB to target an important childhood commensal, suggesting a possible requirement for post implementation surveillance to monitor any potentially detrimental effects of 4CMenB on normal nasopharyngeal flora.

SOP 1: CONJUGATED VACCINE CANDIDATE AGAINST *NEISSERIA MENINGITIDIS*

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Introducción: Meningoccocal disease is one of the must important causes of meningitis. Estimates from WHO indicates that every year occurred more than 1 million of invasive cases and 100 000 deaths. The situation is more difficult in African countries from the "meningitis belt" where epidemics from serogroups A, W135 affect entire region. Conjugated polysaccharide vaccines are more immunogenic, induce long lasting immune response, memory and herd immunity compared with plain polysaccharide vaccines. However, conjugated vaccines are more expensive and less affordable to poor countries. Finlay Institute in collaboration with the Center of Biomolecular Chemistry (CQB) aims to develop an affordable multivalent conjugated vaccine candidate against *N. meningitidis.* **M&M:** Polysaccharides were conjugated to diphtheria toxoid and evaluated as tetravalent formulation. A dose response study was carried out in BALB/c mice with the conjugate formulation and compared to commercial vaccines. **Results:** Conjugates of serogroups A, C, Y, W135 of *N. meningitidis* were formulated and the dose response study demonstrated that high antibodies level and bactericidal activity were induced, comparable with the commercial vaccine Menveo. **Conclusions:** Evaluation of tetravalent conjugate formulation during experimental stage was successful. The development of the multivalent vaccine candidate is ongoing.

PROGRESS AND PERSPECTIVES OF MENAFRIVAC, A MENINGOCOCCAL A CONJUGATE VACCINE FOR THE AFRICAN MENINGITIS BELT

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Introduction and aim: Recurrent group A meningococcal epidemics is a major public health problem in the African meningitis belt. The Meningitis Vaccine Project is a partnership between the WHO and PATH, funded by the Bill & Melinda Gates foundation, with the mission to eliminate epidemic meningitis as a public health problem in Africa through the development, testing, licensure, introduction, and widespread use of affordable meningococcal conjugate vaccines. **Results:** Following international standards, an affordable monovalent group A conjugate vaccine, MenAfriVac, was developed through a public private partnership. MenAfriVac had Indian market authorization (December 2009), WHO prequalification (June 2010) and was introduced through vaccination campaigns with a single dose among 1- to 29-year-olds. Between 2010 and 2012, ten countries launched their national campaigns, and over the past two epidemic seasons, there have been a dramatic fall in cases of group A meningococcal

disease in these countries. As at the end of 2012, there have been no reports of cases of Men A disease in more than 100 million vaccinated individuals. Also MenAfriVac received regulatory approvals to be used in a control temperature chain for up to 40°C for 4 days. This was piloted in Benin in 2012 and preliminary safety results suggest that this could be the solution to inadequate cold chain facilities. **Conclusion:** The high vaccine coverage achieved in campaigns augurs well for rollout in the additional 16 countries that constitute the African meningitis belt. Continuing surveillance and monitoring of vaccination coverage and safety are necessary to confirm the effects of the vaccine. An important challenge is how to protect new birth cohorts.

A TRIVALENT OUTER MEMBRANE VESICLE (OMV) VACCINE AGAINST MENINGOCOCCAL DISEASE FOR AFRICA

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Introduction: The recent decade has demonstrated that there is a need for a vaccine to cover meningococci of serogroups A, W-135 and X to prevent most epidemic meningitis in the African meningitis belt. The current study explores the serogroup X specific immunogenicity in mice induced by combining X-OMV or X-polysaccharide (X-PS) with a serogroup A+W-135 OMV vaccine. **Methods**: Groups of NMRI mice were immunised with two doses of either a combination of the A+W-135 OMV vaccine with X-OMV or, X-PS or each of the monovalent vaccine products. The OMVs were derived from wild-type disease isolates of serogroups A (strain Mk499/03), W-135 (strain Mk222/02) and X (strain BuFa2/97); for which the sequence types (STs) were 7, 11 and 751, respectively. Serum bactericidal activity (SBA) was evaluated using the homologous vaccine strains with rabbit complement. **Results**: Immunisation of mice with X-OMV, either alone or in combination with A+W135 OMVs induced serum bactericidal antibodies against the serogroup X target strain BuFa2/97; even after one dose. A combination of X-PS with the A+W-135 OMV vaccine did also induce high SBA titers against the group X-strain, whereas the X-PS alone was not immunogenic in mice. Comparable SBA titers against serogroup A (vaccine strain) were induced in mice immunized with A-OMV as a monovalent vaccine or combined with W-OMV and X-PS/X-OMV. Strong OMV specific antibody responses were induced against the corresponding OMVs, and a relatively low level of cross-reaction between serogroups was observed. **Conclusion**: Both serogroup X OMV, or a mixture of X-PS or X-OMV with A+W135 OMVs, were shown to induce putatively protective antibodies against serogroup X meningococci. Moreover, addition of X-PS or X-OMV to an A+W135 OMV vaccine did not appear to decrease the immunogenicity against serogroups A or W-135. Thus, a trivalent AXW-135 vaccine, either as a combination of OMVs or OMVs and X-PS, may be able to prevent the majority of meningococcal disease in the African meningitis belt.

SOP 2: AN APPROACH TO TETRAVALENT VACCINE PRODUCTION FROM POLYSACCHARIDE OF N. MENINGITIDES SEROGROUPS ACYW135, TO MUSLIM MARKET

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Introduction: Meningococcal disease continues to be a significant global concern, with an estimated 1.2 million cases and 135000 deaths annually. Polysaccharides vaccines are shown to be efficacious in children and adults, but the efficacy is limited in infants and young children; on the other hand, the conjugate vaccines are too expensive and not reachable by poor countries, been widely used single

polysaccharide vaccines to epidemic control in many countries, helping to save lives. Muslim people are an interesting market segment, so vaccines are direct to reach those populations. A technology update, in order to get higher polysaccharide expression in fermentation with shorter purification steps, to obtain a competitive product fulfills the Halal requirements. The **Aims** of this work is to develop a technology to obtain capsular purified polysaccharide from *Nm*. serogroups $ACYW_{135}$ focus on Muslim vaccines formulation. **Methods**: New strains, with traceable story were evaluated; raw material HALAL or non animal origins certified were used in a concentrated media, a cetrimide precipitation with selective ethanol extraction in purification steps was archive. Get a new facility; which meets the cGMP, dedicated and a System of Quality Assurance for HALAL productions. **Results:** The strains and raw materials get high polysaccharide expression; the polysaccharides purified fulfill the specifications. **Conclusion:** The new process allows obtaining a polysaccharide according to the Muslim requirements.

SOP 3: NEW VACCINES STRATEGIES AGAINST *NEISSERIA MENINGITIDIS* SEROGROUP X

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Introduction: Most meningococcal disease in Africa is caused by serogroups A and W-135 of N. meningitidis. Recently, new cases of meningitis caused by *N. meningitidis* serogroup X have been reported in countries from "meningitis belt". No vaccines have been developed against this serogroup.. The aim of this work is to show the different R&D strategies under evaluation in Finlay Institute against the pathogen. Materials and Methods: Experimental lots of OMVx were obtained by deoxycholate extraction method from N. meningitidis serogroup X BuFa 2/97 strain. Physico-chemical characterization was carried out to determine the size, morphology and the main antigens in vesicles. In addition, capsular polysaccharide X (PsX) was obtained by phenol free process and characterized by HPLC, HPAEC-PAD and other analytical techniques. OMVx were adsorbed to aluminum hydroxide (OMVx/AL) and were administered alone or in combination with PsX. Antigen specific IgG responses induced by these formulations to polysaccharides or OMVx were evaluated by ELISA, and serum bactericidal assay (SBA). Results: OMVx size was between 90-120 nm and OpcA, PorA and RmpM protein were identified. Lots from PsX were obtained by high scale process (100 L). PsX size was estimated in 500 g/mol L and Kd in 0.5. OMVx/AL induced high specific anti-OMVx antibodies response in sera with bactericidal activity. OMVx with PsX also contributes to increase SBA in the group of mice immunized with this formulation as well as the induction of anti PsX antibodies. Conclusion: Development of novel vaccine candidates against serogroup X is under evaluation. Combination of OMVx with the PsX as well as the formulation of multivalent ACYW135 and X plain polysaccharide vaccines could represent a viable solution to meningococcal disease in Africa.

Tuesday, May 21, 2013

Symposium IV: Gonococcal vaccine

Chairs: P. Rice (USA) and L. Velásquez (Chile)

KNA-7: SACCHARIDE ANTIBODY PROTECTION AGAINST *NEISSERIA GONORRHOEAE* INFECTIONS IN THE EXPERIMENTAL MOUSE MODEL CAN REVERSED BY ANTI. RMP ANTIBODY. This conference will include both abstract below

PROTECTION AGAINST VAGINAL COLONIZATION WITH *NEISSERIA GONORRHOEAE* IN A MOUSE MODEL BY PASSIVE (2C7 MAB) AND ACTIVE IMMUNIZATIONS USING A PEPTIDE SURROGATE OF THE 2C7 LOS EPITOPE

Gulati[1] S, Xin[1] M, Zhang[1] B, Reed[2] G.W, Ram[1] S and Rice PA*[1]

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The emergence of ceftriaxone-resistant strains of *N. gonorrhoeae* may herald an era when gonorrhea will be untreatable with conventional antibiotics. There is an urgent need to develop vaccines against this infection. The 2C7 epitope is a conserved oligosaccharide (OS) structure, a part of lipooligosaccharide (LOS) on *Neisseria gonorrhoeae*. The epitope is expressed by 94% of gonococci residing in the human genital tract (*in vivo*) and may represent a potential candidate for an anti-gonococcal vaccine. To circumvent the limitations of saccharide immunogens in producing long lived immune responses, previously we developed a peptide mimic (called PEP1) as an immunologic surrogate of the 2C7-OS epitope by selecting candidate peptides from a peptide library using mAb 2C7 and reconfigured one of these into a multi-antigenic form (MAP), called MAP1. To test efficacy of MAP1 as a vaccine candidate, female BALB/c mice were immunized either with MAP1 or an irrelevant MAP control (called MAP2) together with monophosphoryl lipid A (MPL) used as adjuvant. Mice immunized with MAP1 developed a T_H1 biased anti-LOS IgG antibody response that together with human complement was bactericidal; immunization with the MAP2 control peptide did not yield an anti-LOS response. Immune mice were challenged with live *N. gonorrhoeae* in 2 separate experiments. Median times to clearance were 5 days in the MAP1 groups (n=24 mice in the two experiments) vs 9 days in the MAP2 controls (n=21 mice) ($p=0.0001$ and $p=0.0002$, respectively). Bacterial burden over the course of infection was also lower in MAP1 immunized mice ($p\leq0.0001$).

ANTIBODY TO REDUCTION MODIFIABLE PROTEIN (RMP) INCREASES THE BACTERIAL BURDEN AND THE DURATION OF GONOCOCCAL INFECTION IN A MOUSE MODEL

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Neisseria gonorrhoeae possesses several mechanisms to subvert human immune defenses. A previous study (Plummer FA et al, J Clin Invest. 1993; 9: 339-43) showed that female commercial sex workers in Kenya whose sera contained antibodies (Abs) directed against Rmp had a higher risk of acquiring repeated gonococcal infection (OR = 3.4). Anti-Rmp antibodies block the killing effect of bactericidal Abs directed against gonococcal lipooligosaccharide (LOS), such as mAb 2C7, an antibody directed against a common LOS epitope. We tested the efficacy of mAb 2C7 in clearing gonococcal vaginal colonization in mice that possessed anti-Rmp Abs resulting from: (1) active immunization with purified recombinant gonococcal Rmp (rRmp) or (2) passive transfer of affinity-purified mouse polyclonal anti-

Rmp antibodies. Time to clearance showed that the control group receiving only mAb 2C7 cleared gonococci significantly faster than mice actively immunized with rRmp or mice given anti-Rmp Ab; both groups having been administered mAb 2C7 (median times to clearance 7 and 8.5 days respectively vs. 4 days in control animals administered mAb 2C7 alone; $P<0.0001$). The 'area under the curve' (AUC), a reflection of bacterial burden over the entire duration of the experiment, was calculated for each animal. AUCs for the "2C7-alone" group was significantly lower than the AUCs of each group of mice that possessed anti-Rmp Ab in addition to having been administered mAb 2C7 ($P\leq0.006$ in both instances). Anti-Rmp Ab blocks the disease attenuating effects of mAb 2C7 in the mouse gonococcal vaginal colonization model. Pre-existing anti-Rmp Ab will be an important consideration in evaluating the efficacy of gonococcal vaccines that may protect via a complement dependent antibody mechanism.

ROLE OF GLYCOCALYX AND MATRIX EXTRACELLULAR PROTEINS IN THE PATHOGENESIS OF *NEISSERIA GONORRHEAE* ON FALLOPIAN TUBE CELLS

Velásquez L.

Pending Abstract

SURFACTANT VESICLES AS A SURROGATE FOR WHOLE CELL VACCINATION ALLOW FOR INCLUSION OF LIPOOLIGOSACCHARIDE IN VACCINE PREPARATIONS

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Introduction: To date, no one has been able to develop a simple whole cell-based vaccine for neisserial infections due to the inherent toxicity of lipooligosaccharide (LOS). **Aims:** Development of a gonococcal vaccine. **Results:** We have demonstrated that this toxicity can be circumvented by extracting cell surface components into negatively-charged catanionic vesicles. We have developed this vesicle technology to allow for simple extraction of outer membrane components. Our data indicates that the surface antigens expressed by the gonococcus are stably maintained in these vesicles, are expressed on the surface of these vesicles, and are capable of eliciting a strong anti-gonococcal antibody response. Catanionic surfactant vesicle formulations are stable at room temperature for years and can be sterilized by simple pasteurization. Intraperitoneal immunization of mice with catanionic surfactant vesicle formulations produced no observable adverse effects in mice. This is contrasted with intraperitoneal immunization of mice with equivalent amounts of purified LOS induced significant adverse effects. A strong immune response directed against both protein and LOS antigens was observed. Our surfactant vesicle platform possesses all of the advantages seen with traditional liposome formulations, without any of the inherent problems associated with liposome-mediated vaccines. This vaccine platform readily lends itself to further modifications in that it is possible to include additional neisserial proteins into the vaccine via supplementation with cloned genes purified from other hosts, or by the incorporation of immune modulators into the vesicles. **Conclusions:** We believe that this platform will allow us to generate a universal vaccine able to protect against all serotypes of *N. meningitidis.*

RNA-SEQ ANALYSIS OF VAGINAL LAVAGE SAMPLES FROM FEMALE PATIENTS IDENTIFIES A REPERTOIRE OF PUTATIVE GONOCOCCAL VACCINE TARGETS

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Gonorrhea, the STI caused by *Neisseria gonorrhoeae*, represents the second most common reportable disease in the U.S with over 320,000 confirmed cases in 2011 and as many as 700,000 estimated cases (Centers for Disease Control). Infection by *N. gonorrhoeae* is becoming particularly worrisome, as the number of antibiotic resistant strains of N. gonorrhoeae has been on the rise. In addition, there is no reliable vaccine for the disease due to a variety of reasons including antigenic variability and lack of consistent expression of commonly used antigens across strains and conditions. Identification of new vaccine targets is complicated by the lack of a global analysis of the *N. gonorrhoeae* transcriptome during infection. Here we present the first such analysis of the transcriptional response of *N. gonorrhoeae* during natural infection of the female genital tract. Vaginal lavages were obtained from female patients visiting the NCSTD clinic in Nanjing, China. RNA was isolated and sequenced before being aligned to several sequenced gonococcal strains to determine individual gene expression levels. All gonococcal isolates analyzed aligned best to the TCDC-NGO8107 strain first isolated in Taiwan. We detected expression of 1576 gonococcal genes during infection as well as 48 other instances of transcription not aligning to any known gene. The most highly expressed genes include those involved in stress response and pilin generation. In addition, outer membrane proteins were very highly expressed, as were several hypothetical proteins. It is possible that these may represent previously uncharacterized putative vaccine targets. We also detected a core set of gonococcal genes that were highly expressed regardless of the particular strain or infection conditions suggesting that the results of this study could be applied to a broad range of gonococcal infections. These studies are the first to analyze the global transcription of *N. gonorrhoeae* during natural clinical infection and show that, among known genes, there are several previously uncharacterized gonococcal genes expressed and that the global transcriptional response shows strong similarity among disparate infection conditions. It is possible that one or more of these expressed genes could serve as strong candidates for a future gonococcal vaccine.

Wednesday, May 22, 2013

Round Table: 25 years of the Proteoliposome as Human Cuban Vaccine

Chairs: D Cardoso (Cuba) and E Rosenqvist (Norway)

VA-MENGOC-BC®: LANDMARKS OF A VACCINE.

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The Antimeningococcal Vaccine BC of Finlay Institute, Vamengoc BC®, is based on purified Outer Membrane Proteins from a carefully selected Group B Vaccine strain, assembled with phospholypids as Proteoliposomes, and co-formulated with Group C polysacharide, resulting a very stable and consistent bivalent BC vaccine. The protective response of Vamengoc BC® is based on specific antibodies directed to OMPs and LPS, with opsonic and bactericidal activity and a dominating Th-1 cytoquine pattern, as well as a long lasting immunological memory. This vaccine was massively used in Cuba during the 80s to control a very severe epidemic due to men B and has been from that time included in the National Immunization Program; Many other countries, mainly from Latin America, applied Vamengoc BC to control outbreaks and epidemics with more than 80 million of applied doses in Cuba and other countries. 25 years of experience with this vaccine have certified its efficacy and safety. From the OMPs vaccine generation VAMENGOC BC was the first and more massively applied to control meningococcal diseases. We are going to present briefly the landmarks of VAMENGOC BC vaccine, it`s scientific basis and development, the clinical safety & efficacy demonstration, the scale up of the production process ,the useful demonstration of the protective capacity of the IMMUNGLOBULINS obtained from vaccines plasma to treat infected children, some other interesting issues concerning scientific and human aspects of this scientific result will be also present honoring the contribution of this vaccine as basis for future developments. Other members of our team will go in more details to specific scientific-technical issues.

CONTROVERSIES REGARDING CLINICAL USE OF VA-MENGOC-BC®

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Introduction. VA-MENGOC-BC® has been the only licensed vaccine against serogroup B *Neisseria meningitidis* for many years. However, some specialized journals have declared that effective B meningococcal vaccines have not been developed yet. On the other hand, some authors state that this vaccine does not provide protection in children less than 4 years of age. **Aims.** To clarify the immune response elicited by VA-MENGOC-BC®, and its influence on the meningococcal disease morbidity. **Results.** VA-MENGOC-BC® was licensed after successful clinical trials that showed a proper immunogenicity, as well as an efficacy of 83%. This vaccine induces a T-helper 1 pattern with proper bactericidal and opsonophagocytic activity, among other immune mechanisms. Protective response against different *Neisseria meningitidis* serogroup B strains has been detected, even in infants. This vaccine also induces strong immune response against serogroup C meningococci, which support the adjuvant capacity of the proteoliposome. The induction of immunologic hyporesponsiveness has not been demonstrated. It has been successfully used for epidemic control in several countries. After mass vaccination campaigns there was a rapid fall in the incidence rates of meningococcal disease in the vaccinated age groups. The incidence rates of this disease in Cuba remains under 0.1 x 100,000

inhabitants during last years. **Conclusions:** 1) VA-MENGOC-BC® induces an evident protective immune response against both serogroup B and serogroup C strains in infants, children and adults. 2) The immune response elicited by this vaccine is not completely restricted strain-specific. 3) Immunologic hyporesponsiveness has not been proven.

VA-MENGOC-BC®: 25 YEARS OF SECURITY AND EFFICACY PRECLINICAL TRIALS

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Introduction. Meningococcal meningitis caused by the Gram-negative bacteria *Neisseria meningitidis* is a disease of sudden occurrence, quick evolution and fatal prognosis. The level reached by the pharmaceutical industry requirements has imposed the need to obtain and develop new and better experimental biomodels in order to determine the toxic effects and to establish the adequate rate of cost-benefits of vaccines. **Aims**. The goal of this work is to summarize and analyze the results of numerous preclinical trials designed to establish the security and efficacy of the Cuban meningococcal BC vaccine (*VA-MENGOC-BC®*) during 25 years. **Results**. A summary of the preclinical trials including the development and characterization of biomodels using different species (monky, mice and rat) as well as the results of the use of these models for the evaluation of immunogenicity, local tolerance, toxicity (one and repeat doses), and protection selected to evaluate the security and efficacy of the vaccine are included. Also were carried out studies of indirect protection using the Finlay Institute´s proprietary Gammaglobulin anti-meningoccic BC. It was evidenced during the different preclinical trials with the vaccine, that mice is the best biomodel to evaluate protection whereas rat is the biomodel of choice to evaluate safety and the use of monkies produced expanded insight into safety issues. **Conclusion**. The results obtained in the pre clinical evaluation of *VA-MENGOC-BC®* through 25 years shows clearly and confirm the proved security and efficacy of this vaccine.

POST-LICENSE SURVEILLANCE OF VA-MENGOC-BC®

Cuevas I

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Introduction. During the phases of clinical development, the vaccine's safety evaluation were strictly followed, and the same after their introduction in the market. **Aims.** To show the vaccine's safety profile during the post-license surveillance of VA-MENGOC-BC®. **Results.** The Phase I trial (1985) conducted in adult volunteers, have indicated that adverse effects were limited to a rise in body temperature to 99.5°F (37.5 ºC), and slight pain at the injection site – similar to that produced by the tetanus vaccine. The Phase II clinical trial with children aged 6 months to 12 years, had similar result and no fever was observed. In a Phase III safety trial, results showed discrete predominance of local symptoms (56%) over general (44%). The most important were induration, redness, and pain at the injection site. In 1991, the vaccine was included in the National Immunization Program: a retrospective study (Havana City, 1998) showed that adverse effects detected were similar to those described above, and they all disappeared within the first 72 hours post-vaccination. In Brazil (1990-1997), the vaccine was considered of slight reactogenicity and well tolerated. In Argentina, active observations were carried out during the vaccination campaigns using VA-MENGOC-BC® in adults, revealing that most reactions observed were local. In Uruguay, the vaccine showed that the 71% of the events were local reactions. **Conclusion.** Over 55 million doses of the vaccine have been administered with rare serious adverse

effects (anaphylactic shock, hypotonic hypo-responsive episodes, angioneurotic edema): fewer than 1 per million doses administered.

TECHNOLOGICAL PLATFORM FOR VA-MENGOC-BC® PRODUCTION

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Introduction. Finlay Institute has a technology for VA-MENGOC-BC® obtainment that has provided a consistent manufacturing response to both national and international requests. **Aims.** To present the technological bases for the obtainment process of VA-MENGOC-BC® and the established industrial platform within an improvement process motivated by the increase in latest regulatory manufacturing standards. **Results.** The formulation of the vaccine is based on the solubilization of the outer membrane vesicles (OMV) from *N. meningitis* disserogroup B in the serogroup C meningococcal capsular polysaccharide (PC-C) and the obtained complex is adsorbed onto Aluminum hydroxide. Thus, more than 60 millions doses have been consistently obtained. Active pharmaceutical ingredients (API), OMV and PC-C are obtained by the application of a system of seed lot for culture production, followed by stages of harvest, capture and purification of bacterial subunits. Production flow charts and controls of main stages are shown as well as their relation to API quality specification compliance. The methodology used in the evaluation of an improvement change is provided to demonstrate it does not affect the quality characteristics of the product and its stability. The general characteristics of the production plants and their flexibility for campaign production of the products are also presented. **Conclusions.** More than 60 millions doses of VA-MENGOC-BC have been consistently manufactured using a technological platform that has permitted to comply with new regulatory standards and has made easy the introduction of other products.

COMMERCIAL EXPERIENCES OF VA-MENGOC-BC®

Caro E

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VA MENGOC BC® is the first vaccine with proven efficacy against meningococcus group B. It arrives to twenty five years of massive application. It was registered in Cuba in 1987, and included in the National Immunization Program in 1991. It is indicated for active immunization of infants, children, adolescents and adults. At present it has been registered, or is in registration process in more than 15 countries, while it is patented in twenty. More than 65 million doses have been applied, and its impact has been evidenced in Cuba and other Latin-American countries such as Brazil, Uruguay, Argentina and Colombia against meningococcal diseases caused by serogroup B (homologous and heterologous strains) and serogroup C in massive campaigns. Based on these observations we conclude that VA-MENGOC-BC® provides protection and it is effective in epidemics not only with strains homologous to those of the vaccine, but also with the disease caused by other epidemic serotypes of serogroup B.

PROTEOLIPOSOME AS THE CORE OF VA-MENGOC-BC® AND ADJUVANT PLATFORM

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Introduction. VA-MENGOC-BC® contains OMV (Proteoliposome, PL) and C polysaccharide from *Neisseria meningitidis* serogroup B and C, respectively and alum. It was introduce in the Cuban vaccination schedule since 1991 at infant age. As alum is not a potent adjuvant we hypothesized that PL could has adjuvant activity. In addition, no mucosal adjuvant has been licensee in human vaccine. **Aims.** To demonstrate the adjuvant effect of AF (Adjuvant Finlay) PL1; to develop AFCo (Cochleate) a PL- and Lipopolysaccharide (LPS)-derived mucosal adjuvants; to characterized their mechanisms of actions; and to extend their use to other microorganism, human vaccines and fish. **Results.** AFPL1 (nanoparticle) and AFCo (microparticle) were tested against a panel of model and vaccine antigens. They induce a preferential Th1 polarization including in human with the induction of specific IgA by mucosal routes. AFCo1 (from *N. meningitidis* B PL), AFCo2 (from *Vibrio cholera* PL), and AFCo3 (from *N. meningitidis* B LPS) were obtained. The main MAMP (microbe-associated molecular pattern) associated with them are LPS (TLR4), PorB (TLR2), and DNA traces (TLR9), but LPS was the predominant one. AFPL1 has been also evaluated in a therapeutic/prophylactic allergen vaccine shifting the allergen-specific Th2 pattern to Th1/Tr1 in mice and a Phase I trial is ongoing. AFCo1 is at development stage. AFCo3 has been tested as immunopotentiator in fish decreasing their mortality. AF are able to function in simultaneous primes by parenteral and mucosal routes, inducing also mucosal immune response. Lastly, AF overcomes the thymus-independence of plain bacterial polysaccharides inducing a Th1 polarization and memory immune response at least in mice. **Conclusion.** AF are very promising adjuvants for unsolved-vaccine diseases including mucosal approaches and therapeutic vaccines.

Wednesday, May 22, 2013

Symposium V: *Streptococcus suis*: disease and future challenges

Chairs: V. Verez (Cuba) and M. Gottschalk (Canada)

OVERVIEW OF *STREPTOCOCCUS SUIS* EPIDEMIOLOGY OF THE DISEASE AND VACCINE UPDATE

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Infections caused by *Streptococcus suis* are considered a global and an economical problem in the swine industry, since it is an important aetiological agent of meningitis, septicemia, arthritis, endocarditis and pneumonia in young pigs. Moreover, *S. suis* is an agent of zoonosis that afflicts people in close contact with infected pigs or pork-derived products. In countries where backyard types of production are popular (Asia, some countries in Latin America), the general population is also at risk. Although sporadic cases of *S. suis* infections in humans (mainly meningitis) had been reported during the last 40 years, outbreaks due to this pathogen emerged during the last years in Southeast Asia. The severity of the infection in humans during the outbreaks, such as a shorter incubation time, more rapid disease progression and higher rate of mortality, attracted a lot of attention from the scientific community and the general press. Among the 35 capsular types described, serotypes 2 and, to a certain extent, serotype 14 are mainly implicated as zoonotic agents. In some countries (China) a new highly virulent clone has been described. However, in other countries, more traditional strains were responsible for such outbreaks. In fact, *S. suis* has been found to be the first cause of adult meningitis in Vietnam and the second one in Thailand. Control of the disease in humans should mainly be based on the control of the infection in pigs. In this regards, traditional whole cell bacterins have been used so far with poor results. The development of new generation vaccines for pigs and, probably, for humans is warranted.

MOLECULAR TYPING OF *STREPTOCOCCUS SUIS* FROM PIGS IN CUBA

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Streptococcus suis is a bacterium commonly carried by pigs in the respiratory tract; however, the infections caused by virulent strains are considered a problem in the swine industry. *S. suis*infections can be the cause of meningitis, polyarthritis, polyserositis, septicaemia, pneumonia leading to significant economic losses in pig farms. This bacterium is also a zoonotic agent responsible for meningitis and arthritis in humans in close contact with infected pigs. A successful approach for the identification of virulent strains is the differentiation of capsular serotypes using specific antisera or the corresponding *cps* types by genotypic assessment, with the subsequent detection of virulence associated factors, namely the extracellular factor (EF, ef), the muramidase-released protein (MRP, mrp) and the hemolysinsuilysin (SLY, sly). Data regarding serological and molecular identification of *S.suis* from pigs are not available in Cuba. The objective of this study was thedetection of the capsular types*cps*, as well as three genes related to virulence using PCR assays. Results showed that among the 31 isolates evaluated,these were classified as *cps*2 (n=21) or *cps*9 (n= 4), while the *cps* 7 genotype was not found. Considering the

presence in these isolates of the genes sly, epf and mrp, six different genotypes were differentiated among the *cps*2 or 9 positive strains and three genotypes among the nontypable strains. The *cps2* isolates were recovered from pigs between 6-12 and 14-17 weeks with pneumonia and systemic infection respectively, whereas the *cps 9* isolates were exclusively associated with pneumonia. The genotypic identificationwas confirmed byassaycoagglutinationusingreference antisera. So far, this is the first study on the serological and molecular identification of *S. suis*isolates in Cuba from disease cases in pigs, indicating the occurrence of different capsular types and nine different genotypes among the *S. suis*isolates. A larger scale sampling among pig farms from different Cuban regions could enable us to design a strategy to minimize the risk of exposure to *S. suis.*

THE IMMUNE RESPONSE AGAINST *STREPTOCOCCUS SUIS*: SEPSIS AND TOXIC SHOCK

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Streptococcus suis is a major swine and human pathogen causing meningitis, acute septicemia and septic shock. The emergence of a hypervirulent clone leading to an unusual streptococcal toxic shock-like syndrome (STSLS) in humans in Asia highlighted our lack of knowledge on the immunopathology of *S. suis* infection. Using cellular and molecular immunological approaches we revealed that *S. suis* employs an arsenal of virulence factors to modulate the functions of dendritic cells (DCs) and consequent activation of both natural killer cells (NKs) and T cells. *Ex vivo* and *in vivo* analysis revealed involvement of CD4+ T cells in the release of signature Th1 cytokines. However, the capsular polysaccharide, one of the major *S. suis* virulence factors, was shown to interfere with T cell activation and impaired antibody responses. This interference correlated with presence of clinical signs in infected mice. A whole genomic approach was then undertaken to understand modulation of host genes that might be crucial during the initial step of the host immune response to an infection caused by *S. suis* strains with different virulence degrees, especially the highly virulent Asian clone. We revealed that infection with a low virulent strain resulted in an IFN-β-subjugated, low inflammatory response that might be beneficial for the host to clear infection. In contrast, our data suggest that the highly virulent epidemic strain has evolved to massively activate IFN-γ production, mainly by NKs, leading to a rapid and lethal STSLS. Consequently, the severity and outcome of infections caused by *S. suis* are likely to depend on the ability of host immune mechanisms to control bacterial growth and to limit spreading of the pathogen without causing excessive inflammation.

THE PATHOGENESIS OF *STREPTOCOCCUS SUIS* MENINGITIS

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Streptococcus suis is a serious cause of meningitis in both, pigs and adult humans. Suggested potential routes of entry of the organism are small cuts in the skin, the nasopharynx and the gastrointestinal tract. A high percentage of *S. suis* meningitis patients will develop significant temporary or long-term hearing loss, with or without vestibular dysfunction. High levels of bacteremia will usually lead to central nervous system (CNS) invasion and meningitis. *S. suis* would reach the CNS by either adhesion and invasion (and is some cases, toxicity) of brain-blood brain microvascular endothelial cells (BMEC) and/or choroid plexus epithelial cells (CPEC). Meningitis-associated brain injury and neuronal death would mainly be linked with a host reaction to bacterial components. *In vitro* studies have shown that both BMEC and CPEC, as well as microglial cells and astrocytes, stimulated by *S. suis* release high

amounts of pro-inflammatory mediators. *S. suis* is also able to induce an up-regulation of ICAM-1, CD11c/CD18 and CD11a/CD18 on monocytes as well induce the shedding of ICAM-1 from BMEC, which would lead to increased adhesion of monocytes to endothelial cells, thus contributing to inflammatory features of meningitis caused by this pathogen. *S. suis* cell wall components have been mainly involved in cell activation. In fact, as shown for other streptococci, soluble peptidoglycan/cell-wall fragments (including LTA) may be released by exponentially growing bacteria as well as antibiotic treatment (especially β-lactams) and cause a burst of meningeal inflammation. Although not directly implicated in inflammation, the suilysin (hemoysin) may indirectly play a role by releasing hemoglobin, which has been shown to significantly potentiate the induction of pro-inflammatory mediators by acting in synergy with *S. suis* cell wall components.

NEW CHEMICAL METHODS FOR CONJUGATE VACCINES: IS THERE AN AVENUE FOR *STREPTOCOCCUS SUIS*?

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Glycococonjugates to be used as vaccines can be processed by Antigen Presenting Cells (APCs) through a wide range of receptors depending on their size, shapes, molecular diversity, and structural elements. A brief overview of these mechanisms will be presented in light of modern synthetic methods, nanotechnology, and successful applications. A few recent cases will be exemplified using DC-SIGN, ZPS, and Toll-like receptors. The discussion will also succinctly present some tumor associated carbohydrate antigens (TACAs), since they constituted the most challenging carbohydrate derivatives against which effective antibodies could be raised. As such, the creativity of organic chemist to tackle these antigens have offered several new avenues that are now profitably applied to bacterial polysaccharides, including *Streptococcus suis* and other zoonotic pathogens. The exact knowledge of the chemical identity and structures of the carbohydrate antigens can be advantageously taken into consideration for the design of novel chemical entities. This is particularly vital from an intellectual property point of view as the arsenal of existing patents on carbohydrate vaccines tends to preclude novel entries. To overcome the existing limitations in this regards, novel linking and carrier strategies need to be critically developed. This presentation seeks to widen the horizons of existing methods and stimulate useful discussions.

LEARNING FROM *STREPTOCOCCUS PNEUMONIAE* CONJUGATE VACCINE FOR *STREPTOCOCCUS SUIS* MENINGITIS

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S. pneumonia colonize the nasopharyngeal of healthy individual displaying multiple ways for evading the pressure of the host' immunity resulting in a very diverse organism(more than 90 serotypes) with multiple manifestation of disease: meningitis, pneumonia, sepsis and others. The recent development of multivalent glycoconjugate vaccine for preventing disease shows the complexity of this task. *S. suis* is a cause of respiratory disease in pigs and associated with them a zoonotic meningitis in humans. As a bacteria has probably a similar behavior as Streptococcus pneumonia in humans it could be prevented at least potentially by immunization with conjugate vaccines using bacterial capsular polysaccharide of the most prevalent serotypes. Our experience in developing a monovalent glycoconjugate vaccine against *Haemophilus influenzae* type b with a synthetic antigen and a multivalent glycoconjugate vaccine against *S. pneumonia* using the purified capsular polysaccharide will be discussed. Knowledge of epidemiology,

on circulating serotypes, on molecular structure of individual capsular polysaccharides, potential adjuvant and possible combination and formulation of a final product could be among other the basic for the development of a multivalent glycoconjugate vaccine again *S. suis*.

Thursday, May 23, 2013

Symposium VI: Pneumococcal meningitis and Vaccines

Chairs: W. Hausdorff (USA) and V. Verez (Cuba)

KNA 8: EPIDEMIOLOGY OF PNEUMOCOCCAL MENINGITIS, SEROTYPES RESPONSIBLE, AND THE NEED AND PROSPECT FOR PROTEIN VACCINES

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Pneumococcal conjugate vaccines (PCV) are extremely effective in preventing pneumococcal meningitis (PM), both in the infant target population and in older, unvaccinated age groups through herd protection. Nonetheless, with >40 antigenically distinct serogroups (comprising >90 serotypes) of pneumococcus, it was expected that the overall effectiveness of PCVs would be limited to prevention of the 80-90% of PM caused by the 10-11 serogroups they represent. In addition, PCV-7 introduction was followed by complete replacement of the vaccine serotypes colonizing the nasopharynx by non-vaccine types, leading to some replacement disease. It is unclear what proportion of this is actually attributable to PCV-7 use vs. other factors. There is thus interest in a protein-based vaccine to provide broad protection against all pneumococci. The ideal candidate antigens would be expressed by all strains regardless of serotype, be antigenically highly conserved, and represent a virulence or growth factor. Unfortunately, no clinical proof-of-concept has yet been established, and study is complicated by the widespread implementation of highly effective PCVs. Interesting protein candidates include choline binding protein A (CbpA), pneumococcal surface protein A (PspA), pneumococcal surface adhesion A (PsaA), detoxified pneumolysin (dPly) and pneumococcal histidine triad (Pht) proteins. Regarding the latter two, pneumolysin is produced by virtually all pneumococcal strains and is an important virulence factor. The four members of the Pht protein family are well conserved across the pneumococcal species. Immunization with a combined dPly and PhtD formulation protects monkeys from pneumococcal pneumonia. These proteins are currently being studied in a several clinical proof-of-concept studies.

IMPACT OF PNEUMOCOCCAL CONJUGATE VACCINES ON PNEUMOCOCCAL MENINGITIS IN LATIN AMERICA

Di Fabio JL

Pending Abstract

UK EXPERIENCE WITH PNEUMOCOCCAL MENINGITIS AND PCV

Borrow R

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Introduction and aims: In April 2010 PCV13 replaced PCV7 in the UK infant immunisation programme with a 2,4,13 month schedule. This presentation provides estimates of the effectiveness (VE) of PCV13 against invasive pneumococcal disease (IPD) for the additional 6 serotypes in PCV13 plus

6C. **Methods:** The Health Protection Agency introduced enhanced IPD surveillance prior to PCV introduction and obtains additional clinical and vaccination histories for laboratory confirmed cases in vaccine-eligible children. The indirect cohort method, where non-vaccine-type cases serve as controls is used to estimate VE. Adjustment is made for age and period. The impact of differential serotype replacement by vaccination status on this methodology is investigated. **Results:** Adjusted VE for two doses under one year of age for PCV7 (7 serotypes), PCV13 (PCV7 serotypes) and additional 6 serotypes plus 6C was 79 % (24 to 94), 95 % (56 to 99) and 79% (45 to 92), respectively. Effectiveness of the full 2+1 schedule could not yet be assessed. Serotype-specific VE for at least one dose for serotypes 1, 3, 6A, 7F and 19A was 82% (38 to 95), -6% (-158 to 57), 97% (57 to 99), 92% (70 to 98) and 70% (30 to 86), respectively. There was no evidence of an increase in IPD in children <5 years due to all serotypes not covered by PCV13 in 2012/13. In adults aged ≥ 65 years, however, non-PCV13 serotypes have continued to increase into 2012/13. **Conclusions:** PCV13 has high effectiveness against the additional serotypes covered by PCV13 and is expected to have a large impact on disease caused by these serotypes.

IMMUNOLOGICAL CONSIDERATIONS AND LESSONS IN COMPARISONS OF BACTERIAL MENINGITIS VACCINES AGAINST HIB, MENINGOCOCCUS AND PNEUMOCOCCUS

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Bacterial conjugate vaccines were developed to control the severe burden of disease caused by encapsulated bacteria. For the first conjugate vaccine to be licensed in humans (1989), *Haemophilus inlfuenzae* type b (Hib) polysaccharide was conjugated to a carrier protein and was found to be immunogenic and protective when used in young children. All forms of invasive Hib disease including meningitis were prevented by use of the vaccine in infancy. *Neisseria meningitides* group C conjugate vaccines were the next to be licensed (1999) and had a dramatic effect on meningococcal C meningitis when introduced into routine infant immunisation programs. Pneumococcal conjugate vaccines followed (2000) with similar success preventing meningitis caused by serotypes in the vaccine. An outstanding question remains the exact mechanism by which the vaccines protect. All three vaccines have profound effects on nasopharyngeal acquisition and this might be a major mechanism by which meningitis is prevented. Challenges that remain for preventing meningitis include: Maintaining long term protection through to adolescence when immunised in infancy only. This is important in the context of the possible waning of vaccine induced immunity combined with the absence of natural boosting due to little circulation of the pathogen. Replacement meningitis by strains of the bacteria not contained in the vaccine. This has not been observed for Hib or Men C but is more of a problem with the Pneumococcus. Novel strategies, such as non-capsule based vaccines may in the future be required to augment the conjugated capsular polysaccharide approach.

***STREPTOCOCCUS PNEUMONIAE* CONJUGATE VACCINE DEVELOPMENT IN CUBA.**

Valdés Y [1], Santana D[1], García D [1], Villar A[1], González D[2], Paredes B[1], Rodríguez L [1], Ramírez U[2], Chang J[1], Lora J[1], and Verez V[*1]

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The development of a conjugate vaccine against multiple serotypes of *S. pneumonia* is a complex task with numerous challenges. Several years ago, the Center for Biomolecular Chemistry launched a research project for developing a heptavalent vaccine containing the seven serotypes of *S. pneumoniae*

more frequently associated with infection in Cuba and South countries. The candidate vaccine contains 2µg of each capsular polysaccharide 1, 5, 14, 18C, 19F and 23F as well 4µg for 6B - all of them conjugated to tetanus toxoid- and aluminum phosphate as adjuvant. As results of many years of research and development, the technology was established for the production of the seven active pharmaceutical ingredients and of the combined vaccine. Several batches have been produced with the aim to assess their physico chemical properties and to perform the preclinical and toxicological studies, guaranteed by a Quality Assurance System. Last year, the first clinical trial was conducted, demonstrating the safety and immunogenicity of vaccine in healthy young. Presently, we are beginning the clinical trials in infants, with focus on Phase I and Phase II no inferiority; thus paving the way toward first Latin-American custom-designed conjugate vaccine against *S. pneumonia.*

Thursday, May 23, 2013

Symposium VII: Correlates of protection for *Neisseria meningitidis* and *Streptococcus pneumonia*

Chairs: R. Borrow (UK) and D. Medini (Italy)

KNA 9: ISSUES WITH CORRELATES OF PROTECTION FOR QUADRIVALENT MENINGOCOCCAL ACWY GLYCOCONJUGATE VACCINES

Borrow R

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The correlate of protection against meningococcal disease was described in the 1960s in a prospective study in US army recruits where a strong correlation was observed between the development of serogroup C disease and serogroup C serum bactericidal antibody (SBA) titres < 4 as measured using human complement (hSBA) at the time of entry into training. This seminal finding was then backed up by indirect evidence as hSBA titres ≥ 4 were found to be inversely correlated with clinical protection against invasive meningococcal disease. Further information on the correlation between SBA titres and protection against invasive meningococcal disease emerged following the introduction of the monovalent meningococcal serogroup C glycoconjugate (MCC) vaccines in the UK in 1999. Successive evaluations of the correlates of protection for serogroup C disease were performed as data emerged on vaccine effectiveness and the results of SBA testing in which baby rabbit complement (rSBA) was utilised. From the estimates of effectiveness by age group in the UK and the immunogenicity data obtained from clinical trials with three MCC vaccines it was proposed that rSBA titres of ≥ 8 correlated with short-term and long-term protection. Currently there is no consensus regarding the use of hSBA or rSBA and opinion is divided regarding the possible advantages and disadvantages of each. Published data on SBA responses following quadrivalent glycoconjugate vaccination, however, show significant disparities between hSBA and rSBA data especially for serogroup A. These data will be discussed in this overview.

THE PREPARATION AND USE OF IGG-DEPLETED COMPLEMENT IN MENINGOCOCCAL VACCINE ASSESSMENT

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Introduction: The serum bactericidal assay (SBA) using human complement has been established as a correlate of protection for meningococcal disease vaccines. Acquiring human complement suitable for use in the SBA and other immunoassays assessing vaccine-induced immunity can be difficult, due to high levels of antibodies which result from nasopharyngeal carriage of *Neisseria meningitidis* or commensal *Neisseria* species. These cross-reactive antibodies can evoke meningococcal bacteriolysis in the absence of test antiserum. **Aim:** To develop a reproducible method for large scale (300ml) antibody depletion of pooled human plasma using protein G Sepharose chromatography that leaves complement function intact. **Results:** Plasma anti-coagulated with either heparin or lepirudin was compared to serum in a number of immunoassays to establish the optimum initial source of complement. IgG-depleted plasma was assessed for classical and alternative pathway complement activity using radial

immunodiffusion assays and this was compared with pre-depletion plasma. IgM and IgG subclass levels were assessed by ELISA, and IgG was depleted to undetectable levels following antibody depletion of plasma. IgG-depleted plasma retained very high levels of haemolytic activity and gave highly similar SBA titres to complement from a donor with low anti-meningococcal SBA activity. It has also been used successfully in a range of opsonophagocytosis and antibody-mediated complement deposition immunoassays. **Conclusion:** This human complement source has been effective in assessing functional activity to a wide panel of meningococcal serogroups and strains, as well as a variety of isolates of *Bordetella pertussis*, *Haemophilus influenzae* and Group B *Streptococcus*.

EVALUATING THE EFFICACY OF FHBP CONTAINING VACCINES USING HSBA, THE SURROGATE OF PROTECTION

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The importance of fHBP, a lipidated protein utilized by meningococci to evade complement attack, is reflected by the presence of the gene and expression of the protein on the surface of essentially all *N. meningitidis* serogroup B isolates. Antibodies directed to this virulence factor may protect via two mechanisms, inhibiting factor H binding as well as mediating bactericidal killing, and supports inclusion of fHBP in vaccines designed to prevent *N meningitis* serogroup B disease. Given the low incidence of meningococcal serogroup B (MnB) disease, proof of vaccine efficacy in Phase 3 trials must be based upon the serum bactericidal antibody activity using human complement (hSBA). fHBP amino acid sequences from MnB isolates, including invasive disease and carriage, vary. Therefore, to protect broadly, fHBP vaccine components must elicit bactericidal activity that are effective against MnB strains bearing divergent (heterologous) fHBP sequences. Clinical data confirm that fHBP variants fall into two immunologically distinct groups. These observations paired with a comprehensive survey of systematically collected strains allow unbiased selection of epidemiologically relevant MnB strains representative of fHBP diversity, prevalence and expression for evaluation of vaccine efficacy using hSBA.

SERUM BACTERICIDAL ANTIBODY AGAINST CAPSULAR GROUP B MENINGOCOCCAL ISOLATES RESPONSIBLE FOR INVASIVE DISEASE SHOWS THAT MATS IS A CONSERVATIVE PREDICTOR OF STRAIN COVERAGE BY THE 4CMENB VACCINE

Frosi G[1], Biolchi A[1], Lo Sapio M[1], Rigat F[1], Gilchrist S[2], Lucidarme J[2], Findlow J[2], Borrow R[2], Pizza M[1], Giuliani MM[1] and Medini D*[1].

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Background. 4CMenB (Bexsero), a multicomponent protein-based vaccine developed to prevent invasive meningococcal disease caused by capsular group B strains (MenB), was recently approved for use by the European Medicines Agency. Assessment of 4CMenB strain coverage in specific epidemiological settings is of primary importance to predict the impact of vaccination on the burden of disease, afflicting mainly infants and adolescents. The Meningococcal Antigen Typing System (MATS) was developed to predict 4CMenB strain coverage as a surrogate of the Serum Bactericidal Antibody assay using human complement (hSBA), whose use is impractical against large panels of clinical isolates. However, the MATS method was established using hSBA data from a diverse panel of strains not representative of any specific epidemiology. ***Objective.*** To experimentally validate the accuracy of MATS based predictions against strains representative of a specific epidemiologic setting. ***Methods and***

Results. We applied a stratified proportional sampling method to identify an unbiased and representative sample of all MenB disease isolates collected from England and Wales by the Health Protection Agency (HPA) in the 2007–2008 epidemiological year. We tested the selected strain panel in the hSBA assay with pooled sera from both infant and adolescent vaccinees, and compared to MATS predictions. MATS predictions and hSBA results were significantly associated (p-val 0.022) and MATS had a 78% accuracy and a 96% positive predictive value against hSBA results. MATS predicted coverage of 70% (95%CI: 55-85) was largely confirmed by killing in the hSBA: 88% (95% CI: 72-95).***Conclusion.*** We confirm that MATS is an accurate and conservative predictor of strain coverage by the 4CMenB vaccine in infants and adolescents.

CARRIAGE AS AN ENDPOINT FOR LICENSING VACCINES: LESSONS FROM PNEUMOCARR

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An international consultation was convened in March 2012 to provide feedback on the Case for Carriage, a summary statement by the Pneumococcal Carriage Consortium (PneumoCarr) proposing nasopharyngeal (NP) colonization as a supplementary or alternative endpoint in vaccine licensure. PneumoCarr members provided information to vaccine manufacturers, regulators and the WHO on the evidence for NP carriage as a precursor to pneumococcal disease, standardization of laboratory methods for the detection of multiple serotype carriage, definition and estimation of pneumococcal vaccine efficacy against carriage (VE-col), and the direct and indirect impact of vaccination on carriage. Manufacturers and regulators had the opportunity to respond to the information compiled by PneumoCarr and share their perspectives. VE-col as a licensure endpoint may be more useful for the next generation pneumococcal vaccine products, particularly those for which the immunological correlate of protection is not established, whereas it may be less needed for pneumococcal conjugate vaccines which have an established licensure pathway. The consultation supported the importance of NP carriage data as a critical element linking vaccine impact on the individual direct risk of disease to the population-level impact: indirect effects such as herd protection and serotype replacement. The indirect effects of vaccination, however, are not currently established as part of the licensure process and to include them would be a paradigm shift for regulatory agencies who currently consider this information in the post-licensure setting. More discussion and consensus-building is needed around the rationale and optimal mechanism to include carriage data in the licensure pathway for new pneumococcal vaccines. The WHO and national advisory groups on immunization policy may have an important role in considering the evidence for the indirect benefit of vaccination as informed by its impact on NP carriage.

SOP 4: USE OF SERUM BACTERICIDAL ANTIBODY AS AN EVALUATION METHOD FOR IMMUNOGENICITY OF MENINGOCOCCAL VACCINES SEROGROUPS A, B, C, X, W_{135}.

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Introduction: Vaccines and Related Biological Products Advisory Committee on April 6, 2011, concluded that meningococcal vaccine effectiveness should be estimated by serum bactericidal antibodies titer measurement and advised that comparative studies between licensed and investigational meningococcal vaccines, using bactericidal antibody determination, could be the basis for inferring effectiveness of new vaccines, that is why it is necessary to have an established test that allow to obtain

reproducible results comparable with those from other laboratories in the world, dedicated to meningococcal vaccines production. **Aims:** To establish serum bactericidal assay (SBA) for different serogroups causing meningococcal disease in order to evaluate polysaccharide (PS) and outer membrane vesicles (OMV) vaccines. **Results:** Finlay Institute has established serum bactericidal assay for A, B, C, X and W_{135} serogroups using tilt method and an automatic colony counter with baby rabbit serum, as complement source. SBA has been used as control method for release VA-MENGOC BC® lots, for comparing polysaccharides (PS) vaccines from A, C and W_{135} in non inferiority studies, as concept proof for a new PS and OMV candidate serogroup X vaccine in mice and for release lots of an AW_{135} OMV vaccine to be included in a phase I clinical trial in Cuba. This method has been capable to distinguish between positive and negative samples, determine the seroconversion and seroprotection levels and compare different vaccines. **Conclusion**: Serum bactericidal assay can reliably measure functional activity *in vitro* and constitutes an obligatory tool for evaluating the immunogenicity and effectiveness of meningococcal vaccines.

Conferences in Havana for Pediatrician, Immunologists and Microbiologist "William Soler" Hospital April 24, 11 AM

PNEUMOCOCCAL CONJUGATE VACCINES: WHAT HAVE WE LEARNED AND WHAT DO WE HAVE YET TO LEARN

Hausdorff WP

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Three pneumococcal conjugate vaccines (PCVs) have been registered and introduced worldwide since 2000: 7 and 13-valent vaccines whose polysaccharides are conjugated to the diphtheria toxoid variant CRM197, and a 10-valent vaccine whose polysaccharides are conjugated to protein D (from *H. influenzae*), tetanus, and diphtheria toxoids.

Understanding the true public health impact of these vaccines is complex due to the need to assess a variety of invasive and non-invasive clinical manifestations; to assess not only vaccine serotype but also vaccine-related and even non-vaccine serotype disease and carriage; and to assess disease in both vaccine-eligible cohorts as well as those benefitting only from herd protection. To date, careful examination of both randomized controlled trial results as well as post-marketing surveillance analyses with these vaccines has revealed the major impact they can have on sepsis, meningitis, and pneumonia, both in vaccine-eligible and other cohorts. Some open questions remain the magnitude of the impact on AOM, and the extent to which serotype replacement in nasopharyngeal carriage is translated into replacement disease, thereby undermining the net benefit of PCV vaccination.

Intriguingly, longer term post-marketing surveillance studies will be needed to identify if there are substantial differences in public health impact between the two higher valent vaccine formulations, despite obvious differences in serotype composition, conjugation methods, and carrier proteins.

UNDERSTANDING RESPONSES TO POLYSACCHARIDE AND CONJUGATE VACCINES IN INFANTS AND ADULTS

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Encapsulated bacteria (S. pneumoniae, *N. meningitidis*, *H. influenzae* type b) are major pathogens in early life due, in part, to the inability of the immature immune system to make antibodies to the polysaccharide capsule surrounding such organisms. Pure polysaccharide vaccines are thus not useful in preventing infection in early life as they are poorly immunogenic. The reason for the poor B cell response to T independent antigens, such as polysaccharides are still not fully elucidated but have focussed on the immaturity/absence of specialised subsets of B cells in the young.

Natural immunity to encapsulated bacteria however develops with increasing age, in part due to exposure to these pathogens which are carried in the nasopharynx. The identification of pneumococcal polysaccharide specific memory B cells in healthy adults suggests that pre-existing memory has been established and that capsular polysaccharide may have been encountered in a T-dependent fashion.

Converting polysaccharide antigens to T dependent antigens lies at the heart of the success of conjugate vaccines. Such vaccines are highly immunogenic in the very young which illustrates that polysaccharide specific plasma cells and memory B cells can be induced in the very young if the antigens are presented in a T dependent fashion. An ongoing challenge to studying such vaccines at the molecular level in humans, and particularly very young children, is the lack of access to compartments other than blood and the low frequency of circulating, antigen specific B cells. Despite this the direct and indirect effects of conjugate vaccines have had a profound effect on paediatric (and adult) infectious diseases.

POSTER ABSTRACTS

1. FOUR MONOCLONAL ANTIBODIES AGAINST CAPSULAR POLYSACCHARIDES OF *NEISSERIA MENINGITIDIS* SEROGROUPS A, C, Y AND W135: ITS APPLICATION IN IDENTITY TESTS

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Murine hybridoma monoclonal antibodies (MAbs) were produced against the capsular polysaccharide (CPs) of serogroups A, C, W135 and Y meningococci (Men A, Men C, Men W, Men Y) in order to develop immunological reagents for the identification of meningococcal polysaccharides. Each serogroup-specific MAb reacted with the CPs from its homologous serogroup only and did not react with CPs from the other three serogroups. The relative affinity of the four MAbs measured by indirect ELISA revealed that Men C MAb was able to detect 78 ng/mL while Men W MAb detected 9.7 ng/mL. Men A and Men Y MAbs showed clear signals down to 4.8 ng/mL. The application of these MAbs for identity tests was demonstrated by their abilities to correctly identify the CPs from serogroups A, C, W135 and Y in multivalent vaccines through ELISA. The MAbs described here are a very valuable set of tools for study meningococcal polysaccharides vaccines.

2. DEVELOPMENT OF SANDWICH ELISAS FOR DETECTION AND QUANTIFICATION OF CAPSULAR POLYSACCHARIDES OF *NEISSERIA MENINGITIDIS* SEROGROUPS A, C, W_{135} AND Y.

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Introduction. *N. meningitidis* is a gram negative bacteria that has been classified in 13 serogroups. The serogroups A, B, C, W_{135}, X and Y are the causing of infectious invasive diseases. Consistency of production is recognized as an important aspect of vaccine manufacture and *in vitro* assays are required for quality control testing of these products. **Aims.** The present study describes the development of a monoclonal antibody (MAb) based sandwich ELISA to detect and quantify capsular polysaccharides (CPs) from meningococci serogroups A, C, W_{135} and Y. **Results.** The MAbs obtained at Finlay Institute were conjugated to horseradish peroxidase and working dilutions were established. For calibration curves, purified CPs as reference materials were used. The technique was linear over the range 10 ng/ml to 78 pg/ml. No cross-reactivity was observed for any of the serogroups tested. **Conclusion.** these results suggest that MAb-based sandwich ELISA is a rapid and simple test to quantify CPs in plain and conjugate polysaccharide vaccines.

3. LATEX PARTICLES AS POTENTIAL REAGENTS FOR DIAGNOSTIC OF MENINGOCOCCAL DISEASE

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Introduction. Agglutination tests can be used in a qualitative manner to assay for the presence of an antigen or an antibody. Polyclonal or monoclonal antibodies (MAbs) conjugated to latex particles are mixed with the antigen and a positive test is indicated by the agglutination between them. **Aims.** Conjugate four murine MAbs obtained against capsular polysaccharides from *N. meningitidis* serogroups A, C, W_{135} and Y to latex particles of 0.8 μm of diameter and to evaluate them. **Materials and Methods.** We immobilized four MAbs to latex particles and the coupling efficiency was determined. Immunoagglutination, cross reaction and sensibility of coupled latex particles was monitored by the aggregate formation**. Results.** The coupling efficiency of MAbs to latex particles was adequate for all MAbs. All four MAbs were found to react with capsular polysaccharide from the homologous serogroup and did not react with related polysaccharides from the heterologous serogroups. The assays were able to detect up to 10 ng/ml polysaccharide. **Conclusion** We demonstrated that all MAbs conjugated to latex particles could be of great utility to obtain a kit for etiologic diagnostic of the meningoencefalistis bacterial.

4. EVALUATION OF A RAPID TEST FOR THE DETECTION OF *NEISSERIA MENINGITIDIS* SEROGROUP B IN HUMAN SERUM AND WHOLE BLOOD USING A NOVEL AFFINITY BIOSENSOR

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Group B *Neisseria meningitidis* (MenB) is the leading cause of meningococcal disease in developed countries. Diagnosing meningococcal disease early provides important information for initial treatment. Currently, PCR techniques are widely used to detect meningococcal DNA, however this takes one working day to perform. The aim of this study was to evaluate a rapid test for MenB using ELISHA (ELectro-ImmunointerfaceS Heterodoxical Approach) immunosensor technology, which is based on electrochemical biosensors that directly measure the antibody-antigen binding reaction. A specific IgM antibody for MenB was used to construct the biosensors. Two meningococcal strains serogroup C11 (MenC, negative analyte control) and MenB NZ 98/254 were heat-killed and spiked at concentrations of 0, 10^2, 10^4 and 10^6 organisms.ml^{-1} into PBS (Phosphate Buffered Saline), serum and whole blood samples, respectively. Measurements were performed by applying 100μL of sample to the immunosensor surface and incubating for 20 minutes at room temperature. Alternating current impedance scans of a minimum of 2 repeats were then performed between frequencies of 1000Hz to 1Hz using a Perkin-Elmer VersaStat V3 potentiostat. Calibration curves were obtained by subtraction of the responses for specific (MenB IgM) and non-specific (anti-digoxin) electrodes, thereby eliminating non-specific adsorption of sample components. The responses of the immunosensors exposed to NZ 98/254 were found to increase with increasing concentration in PBS, serum and whole blood and no cross-reactivity of the immunosensors was found with MenC strain. The limit of detection was found to be as low as 10 organisms in PBS, serum and whole blood.

5. SDS-PAGE AND DENSITOMETRIC ANALYSIS TO DETERMINE THE CONCENTRATION OF LIPOPOLYSACCHARIDE FROM *NEISSERIA MENINGITIDIS* SEROGRUPOS A, W_{135} Y X

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Introduction. For several years the Finlay Institute in collaboration with the " Norwegian Institute of Public Health" are working in a project in order to obtain a vaccine candidate from outer membrane vesicles (OMV) of *N. meningitis* serogroups A, W_{135} and X. In this project is necessary to establish the specifications of quality related with the lipopolisaccharides (LPS), main cause of the pirogenicity of vaccines. **Aims.** To establish the conditions of the SDS-PAGE to use it as a method to quantify residual LPS in the Outer Membrane Vesicles obtained from *N. meningitidis* serogroups A, W_{135} and X. **Methods.** In the experiments were used gels from SDS-PAGE to 15% and samples of LPS of the different serogroups; these gels were tinted with silver, specific for LPS, and the results were analyzed in a densitometer GS-800 (Bio-Rad) controlled by the program "Quantity One". **Results.** The patron curves of LPS for each serogroups were obtained and was quantified the concentration of residual LPS in the OMV from *N. meningitides.* **Conclusion.** It was established the methodology to determine the concentration of LPS of *N. meningitidis* serogroups A, X and W_{135} in samples of OMV with the SDS-PAGE and the specifications of quality established for this parameter, were confirmed.

6. IDENTIFICATION OF ANTIGENIC COMPOSITION OF OUTER MEMBRANE VESICLES FROM *NEISSERIA MENINGITIDIS* SEROGROUP X

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Introduction. Meningococcal disease is a serious health problem worldwide, it is caused by gram negative microorganism *Neisseria meningitidis*. These bacterias can be classified into 13 serogroups according to the chemical structure of the capsular polysaccharide, however only 6 serogroups have been responsible for the majority of reported meningococcal disease: A, B, C, W135, Y and X. There are vaccines against serogroups A, B, C, Y, W135, but there is no vaccine licensed against serogroup X. **Aims.** The aim of this work is to identify the main antigenic and possibly immunestimulatory components in the Outer Membrane Vesicles serogroup X (OMVx). **Methods.** Protein antigens in OMVx were evaluated by SDS/PAGE with Blue comassie staining and conditions for Two Dimensional electrophoresis (2-D) were set. Additionally, specific silver staining was carried out to quantify lipopolysaccharide (LPS) in the vesicles. Finally, immunotransference assay was performed with specific monoclonal antibodies to identify the LPS immunotype and protein antigens in OMVx. **Results.** The gels and transference membranes were analyzed with a GS-800 densitometer and "Quantity One" software. Four proteins with high and medium molecular weight were observed, being a protein of approximately 40 KDa a majoritary one and 2-D also confirmed this result. LPS was revealed by silver staining and quantified (3,48% of LPS/total protein). Identity of LPS was confirmed by immunotransference as L-3,7,9 and protein antigens like RmpM, Por A 1.5 and OpcA, were identified in the OMVx. **Conclusions.** Important antigenic proteins were identified in the OMVx as well as immunestimulatory LPS.

7. NEW METHODOLOGY WESTERN BLOT TECHNIQUE IN IDENTITY ASSAY. OPTIMIZATION AND VALIDATION

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Introduction Traditionally, immunochemical tests like Western blot are much used for determining Identity. Its application for determining Identity for Meningococcal B Outer Membrane Vesicles (OMV) allows identifying the main antigenic proteins present in the intermediate and final products. However, the current Western used is longer than expected (2 days) for an Identity test. **Aim** The aim of this Paper was to optimize the test for one single day and to validate it under the new working conditions. **Materials and methods** In general the method was carried out as usual. However, times for transference, blocking, incubation of anti-P1, P3 and 70 K monoclonal antibodies and Conjugate at 37 °C were reduced to one, a half, two and one hour, respectively. OMV and VA MENGOC BC vaccine samples were tested and the results were compared regarding the old conditions. For validation, it was performed a Specificity study as required for Identity tests. **Results** There was no difference in the identification of the antigenic proteins both for OMV and the finished product. So, it was possible to get information about the presence of protein bands that are relevant for immunogenicity in children in just one day. At the same time, the successful study for specificity revealed that the identification could be performed under the new conditions in a reliable way. **Conclusions** The modified method allowed to identify the antigenic proteins bands corresponding to the proteins P1,P3 and 70 K in OMV and VA MENGOC BC vaccine in a shorter period of time.

8. CHARACTERIZATION OF DRY POLYSACCHARIDES FROM *NEISSERIA MENINGITIDIS* SEROGROUPS A, C, Y AND W_{135} TO BE USED AS INTERNAL CONTROLS

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Introduction: Finlay Institute is a leader center in the development of vaccines against *Neisseria meningitidis*. In the 90's a clinically active vaccine based on Outer Membrane Vesicle was produced against the serogroup B. Recently, a tetravalent vaccine based on Polysaccharides from serogroups A, C, Y and W_{135} has been developed. To face the quality control of this kind of vaccines, new methods and references should be established. **Aims:** The aim of this research is to obtain and characterize 4 polysaccharides to be used as internal controls in quality control. **Materials and Methods**: The Reference Materials Laboratory formulated 4 different freeze-dried candidates starting from dry purified polysaccharides of *Neisseria meningitides* serogroups A, C, Y and W135. Homogeneity studies were performed and the candidates were characterized according to the intended use by O-Acetyl, Syalic acid and Phosphorous contents and migration time zone by capillary electrophoresis. Stability of these materials was monitored during two years (2011 and 2012). **Results:** All lots were found to be homogeneous. Each sample was assigned with a certified value and uncertainty for the intended purposes. The internal controls were stable for 2 years and it was possible to monitor in a consistent and reliable way the behavior of the test used for the quality control of Meningococcal Polysaccharides. **Conclusion**: Four polysaccharide lots were characterized and are available to be used as internal controls during the quality control and lot release of these polysaccharides.

9. COMPARATIVE RESULTS OF THE INDIVIDUAL POLYSACCHARIDES A, C, W, W135 AND THE TETRAVALENT VACCINE ACWY BY USING PYROGEN TEST

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Introduction: The purified dry polysaccharides of *Neisseria meningitidis* obtained in our Institute are used as antigens in the polysaccharide vaccines. USP monograph establishes the Rabbit Pyrogen test for these vaccines. This test is based on the property of some substances called pyrogens of provoking an

increasing of the body temperature in men and some other laboratory animals like rabbits when they are administered by intravenous route. **Aim**: To compare the endotoxin levels present in individual Polysaccharides and the tetravalent A, C, Y and W-135 Polysaccharide vaccine. **Materials and Methods**: 33 samples of Polysaccharide A, 40 of Polysaccharide C, 20 of Polysaccharide Y, 23 of Polysaccharide W 135 and 8 of Tetravalent Vaccine ACWY were tested by Pyrogen test, as recommended by USP. Certified Rabbits (NZW strain), whose weight was ranging between 1,5 and 1,8 kg were provided by CENPALAB. **Results and Discussion**: The obtained results demonstrated that the experimental Tetravalent vaccine ACWY batches showed a higher presence of endotoxins regarding the individual Polysaccharides. **Conclusions:** The polysaccharide Y could be the major endotoxin contributor to the tetravalent vaccine because the Polysaccharides A, C and W135 did not show an increasing of the body temperature in animals when they were tested separately.

10. PREDICTING SERUM BACTERICIDAL RESPONSES: SENSITIVITY AND SPECIFICITY OF A FLOW-CYTOMETRIC COMPLEMENT DEPOSITION ASSAY.

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Introduction: Serum bactericidal activity (SBA) has long been established as a correlate of protection for capsular polysaccharide and outer membrane vesicle-based meningococcal vaccines, with SBA titres of ≥4 measured using human complement established as providing protection. However, the serum bactericidal assay requires large volumes of sera and can be laborious to perform. For new serogroup B vaccines it is vitally important to determine whether protection will extend to all strains that can cause disease. The limited volume of serum available, particularly from paediatric clinical trials, limits the number of strains that can be assessed. Thus the development of high-throughput functional assays, which require very low volumes of serum, is important to determine the potential effectiveness of a vaccine. **Aim:** To develop a flow cytometric assay measuring antibody-mediated complement deposition. **Results:** The assay uses fixed meningococci and fluorescent-conjugated antibodies to measure deposition of C3b/iC3b and C5b-9 (membrane attack complex) on the surface of *Neisseria meningitidis*. The assay uses 5µl serum per assay, which is considerably less than that required for a standard SBA. We have analysed antibody-mediated complement deposition and SBA with a panel of 40 human sera and 5 diverse serogroup B meningococcal strains and have established a preliminary cut-off value for C5b-9 deposition that will predict SBA. **Conclusion:** We have demonstrated that this flow cytometric assay can predict SBA activity with 65-89% accuracy, depending on strain. To determine the utility of this approach for new vaccines, further analysis with larger panels of sera and strains will be required.

11. BRIDGING OF TWO SERUM BACTERICIDAL ANTIBODY ASSAYS USING RABBIT COMPLEMENT PERFORMED AT THE HEALTH PROTECTION AGENCY AND AT GLAXOSMITHKLINE BIOLOGICALS

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The meningococcal serogroup C (MenC) serum bactericidal antibody assay using baby rabbit complement (rSBA) performed at HPA has been defined as the reference assay. Although HPA has expertise with rSBA testing for serogroups A, W, and Y, no reference laboratory exists for these

serogroups. rSBA assays have also been performed at GSK Biologicals' laboratories, hence a technical bridge between HPA and GSK rSBA assays was performed. We compared rSBA responses to serogroups A, C, W, and Y measured by either HPA or GSK on the same set of serum samples taken at pre-vaccination and one month post-vaccination. Some pre-vaccination samples were depleted of IgG and/or IgM and tested by rSBA assays to better understand the contribution of each isotype to rSBA activity. The rSBA titres measured by GSK and HPA for the four serogroups on post-vaccination samples showed a good level of concordance (agreement >80%) and correlation (r value >0.7). The post-vaccination rSBA activity was mediated by specific antibodies, mainly IgG, induced by the vaccine. In contrast, in pre-vaccination samples, a significant number of samples were positive with GSK rSBA assays, but negative with HPA rSBA assays. Inhibition experiments using homologous meningococcal polysaccharides as competitors confirmed the specificity of the GSK rSBA assays. Depletion experiments demonstrated that removal of IgM induced a decrease in GSK rSBA titre. The data suggested that GSK and HPA rSBA assays for meningococcal serogroups A, C, W, and Y have a comparable sensitivity to vaccine-induced antibodies, but the GSK rSBA assays were more sensitive to naturally acquired antibodies than the HPA rSBA assays. Funding by GSK Biologicals, presented at IPNC2012.

12. A MODEL SYSTEM FOR ASSESSMENT OF OPSONISATION OF MENINGOCOCCI AND COMMENSAL SPECIES

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Introduction: While serum bactericidal antibodies (SBA) are the gold standard for measuring protection induced by natural exposure or immunisation against meningococcal disease, opsonisation of bacteria or blebs containing lipooligosaccharide (LOS) are an additional mode of protection to be considered. **Aims**: to develop a flow cytometric method to assess phagocytosis with the human monocytic cell line THP-1; to assess activity of pooled human serum absorbed with individual strains of commensal species for evidence of absorption of opsonic activity against meningococci. **Methods:** Propidium iodide-labelled bacteria were tested by flow cytometry for ingestion by THP-1 cells of commensal or meningococcal isolates incubated with or without pooled normal serum and aliquots of the pool absorbed with individual strains. **Results**: *Neisseria lactamica* (NL) and *Moraxella catarrhalis* (MC) were more readily phagocytosed than *Neisseria meningitidis* expressing different LOS immunotypes. There was correlation between opsonising and bactericidal activity for most strains tested. Absorption with NL significantly reduced opsonisation of meningococcal immunotype strains expressing L3, L5, L6, and L7. Absorption with MC reduced opsonising activity against L3. Absorption activity varied with individual strains. **Conclusions:** The THP-1 cell line was a reliable model for assessment of phagocytosis, eliminating potentially confounding genetic, maturational or environmental factors. Epitopes on commensal strains absorbed opsonising activity against a variety of group B meningococcal phenotypes, but there is considerable variability in the cross reactive antigens among species and strains. Carriage of the commensal species might induce opsonising antibodies that contribute to protection against meningococcal disease.

13. ESTABLISHMENT OF QUALITY ASSURANCE SYSTEM FOR THE PRODUCTION OF POLYSACCHARIDES FROM *NEISSERIA MENINGITIDIS* SEROGROUPS A, C, Y AND W135 UNDER HALAL REQUIREMENTS

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Introduction: The introduction of vaccines against *Neisseria meningitidis* in the Muslim market implies the implementation of new production and control strategies and a quality assurance system able to meet the requirements of the Good Manufacturing Practices (GMP) and HALAL pharmaceutical-general guidelines for the production and control of pharmaceutical products. As the Institution count on a general quality assurance system in place, the system for HALAL productions should be in agreement with its requirements. **Aim:** To establish the quality assurance system for the production and control of polysaccharides from *Neisseria meningitidis* serogroups A, C, Y and W135 under HALAL requirements. **Results:** The requirements defined were as follow: to use new and dedicated facilities / equipments; requirements of personality, qualification and demonstration of the personnel's behavior; guarantee of the supply of HALAL raw materials with the proper certification from evaluated suppliers; storage capacities separated from the rest of the institute and independent sampling; definition of system mandatory documents such as specifications of quality for strain and final products, RML, procedures, programs, validation protocols with code assignation for assuring traceability and independence on their approval; establishment of particular conditions for the production and quality control and regarding lot release the definition of lot summarized protocols for each component and flows for the releasing process. **Conclusions:** Finlay Institute has a system of quality assurance guaranteeing the production, control and release of polysaccharides from *Neisseria meningitidis* serogroups A, C, Y and W135 under HALAL requirements.

14. DEVELOPMENT AND VALIDATION THE ANALYTICAL METHODS IN POLYSACCHARIDE W135 OF MENINGOCOCCAL VACCINES

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Introduction: In the biopharmaceutical industry the development and validation of the analytical methods are crucial for the safety and quality of pharmaceutical products. Validations studies are designed to provide evidences that methods are able to give the expected results. Validation is part of the quality system ensuring the quality designed for a product. **Aim:** The objective of this work was to develop and validate the Quality Control tests for *Neisseria meningitidis* Polysaccharide W_{135}. **Materials and Methods** The following assays were validated: Syalic Acid and Protein contents, Molecular Size distribution, impurities, nucleic acids, humidity, endotoxin test (LAL) and Pyrogens. The parameters evaluated were Precision (Repetibility and Intermediate Precision), Accuracy, Specificity and Linearity and the design depended on the purpose of each test in study. All data were analyzed through the Coefficients of variation and some other statistical tests performed by using Minitab 15.1 program. **Results**: All the parameters successfully passed the predefined criteria under our work conditions. **Conclusions:** It was demonstrated that all the methods met the parameters required according to the foreseen results. It constitutes a warranty for the quality of the product, its clinical use and commercialization.

15. DEVELOPMENT AND VALIDATION OF METHOD FOR QUANTIFICATION MULTIVALENT POLYSACCHARIDES VACCINES BY CAPILLARY ZONE ELECTROPHORESIS

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Introduction Meningococcal polysaccharides are active components of the vaccine against *Neisseria meningitidis* serogroups A, C, and W135. As both Polysaccharides C and W135 contain syalic acid in their structures, the traditional colorimetric methods are not suitable for quality control of vaccine final lots. The capillary zone electrophoresis (CZE) has proved to be a sensitive tool for the quantification of multivalent meningococcal polysaccharides vaccines. Aim The objective of this work was to develop and validate this analytical method in order to show evidences that the test is reliable enough to produce the intended result within the predefined intervals. Materials and Methods CZE method was implemented in a Capillary Ion Analyzer (Agilent corp.), with DAD at 200nm. It used a fused silica capillary uncoated 50µm (d.i.) and length of 40cm. The data were collected and processed with Chemstation software (Agilent Corp.). The following parameters were assessed: system suitability, accuracy, precision, specificity, linearity and range. Results All the validation parameters fulfilled the predefined criteria. Conclusion The method met the requirements for an electrophoretic method designed for quantifying polysaccharides in vaccines.

16. VALIDATION OF A RI-HPLC ETHANOL DETERMINATION METHOD. CHARACTERIZATION OF THESE IMPURITY IN VA-MENGOC BC™ VACCINE

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Introduction: The determination of residual ethanol is a test that uses a system chromatographic HPLC-refractive index, coupled to a column Aminex HPK-87H (BioRad). This method is used to quantify the presence of this residual solvent in the cuban meningococcal vaccine VA-MENGOC BC ™. **Aims:** The objective of this work was to validate this analytical method and to characterize the level of this impurity in some batches of VA-MENGOC BC ™. **Methods**: For this purpose, three batches of VA-MENGOC BC ™ were used with a residual ethanol content of approximately 0.25, 0.3 and 0.35 % respectively. The following parameters were assessed: system suitability (which are a chromatographic method); accurateness; precision; linearity and range; specificity; limit of quantification and detection limit. In other hands, 120 batches of vaccines was evaluated to determine it's impurity contents. **Results:** The method is rated satisfactory for use in the laboratory taking into account all the parameters validation evaluated meet the requirements for a chromatographic method for detecting traces. The RI-HPLC method permitted the characterization of the residual ethanol content of VA-MENGOC BC ™ between 0,18 and 0,42 %. **Conclusion:** This study showed evidences that the test is sufficiently reliable to produce the intended result within the predefined intervals. The level of this impurity in VA-MENGOC BC ™ was below the internationally accepted limit (0.5%).

17. VALIDATION OF THE STERILIZATION EQUIPMENTS FOR SATURATED STEAM (AUTOCLAVES) USED IN THE MENINGOCOCCAL VACCINES PRODUCTION AT FINLAY INSTITUTE

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Introduction: The validation of the sterilization processes that take place in the autoclaves is an international requirement of Good Manufactured Practices to the meningococcal vaccines production; its impact in the reduction or elimination of the microbiologic contamination risks and in the quality and security of the products. **Aims:** The objective of this work was to show the effectiveness of the sterilization processes that are carried out in some autoclaves used in the production and quality control

at Finlay Institute. **Methods:** The validation process of each autoclave (5) included 3 stages: Installation Qualification: It was verified: documentation, critical spare parts, substances for operation and maintenance, instruments calibration, components systems installation according to plans, makers' recommendations and devices of security. Operational Qualification: It was verified: the personnel's qualification, normalized procedures, control systems, integrity of filters, air tightness of the camera and it were carried out the temperature distribution with empty camera. Performance Qualification: validation of method to evaluate the quality of the biological indicators, temperature distribution with loaded camera, penetration of the heat with loaded camera and microbiologic challenge. **Results:** In the Installation and Operation qualifications were obtained that the equipments are agree with the international validation approaches. In the studies of temperature carried out during the qualification of the operation and the performance (distribution and penetration) was obtained 100% of execution of the validation requirements. **Conclusions:** The autoclaves evaluated, are installed in an acceptable form, they operate correctly, so all of them are considered validated for the sterilization operation / decontamination during the productive processes to obtain meningococcal vaccines.

18. CONCURRENT VALIDATION OF THE PROCESS FOR OBTAINING DRY POLYSACCHARIDE FROM *NEISSERIA MENINGITIDIS* SEROGROUP C.

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Introduction: Concurrent validation of the manufacturing process for production of the active pharmaceutical ingredient (API) is becoming a conditioning requirement for obtaining manufacturing licenses as well as sanitary registrations from regulatory authorities. This study was intended to assess the consistency, reproducibility and quality requirements fulfillment of the process for obtaining the serogroup C *Neisseria meningitidis*' dry polysaccharide. **Materials and methods:** Before conducting this study, calibration of measurement instruments of all equipment involved in the process, qualification of critical equipment according to the manufacturing facilities' Master Validation Plan, and the validated state of critical systems (i.e. purified water, injection water, pure steam, and compressed and clean air) was checked. Based on the manufacturing process' production flux, records were designed for registering all critical variables of each process' step. Records were filled for three consecutive sub-processes simultaneously to the occurrence of individual steps. Recorded data was processed. **Results:** Process' reproducibility, consistency, as well as compliance of all quality requirements, were demonstrated. **Conclusions:** Process for obtaining the serogroup C *Neisseria meningitidis* dry polysaccharide is in a validated state.

19. STABILITY STUDIES OF A *NEISSERIA MENINGITIDIS* SEROGROUPS A AND W_{135} VACCINE BASED ON OUTER MEMBRANE VESICLE

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Introduction: Invasive meningococcal disease constitutes a worldwide health problem. Finlay Institute and the Norwegian Institute for Public Health have a collaboration project for the development of a vaccine based on outer membrane vesicles (OMV) against serogroups A and W_{135} . After adjusting the manufacturing process to the new strains and conditions, industrial scale lots of 100 Liters were included in stability studies according to previously defined Protocols. **Materials and Methods**: Three formulated lots containing different OMV lots from both serogroups were used. Two different stability studies were performed: a 24 months shelf life study at 2 - 8 ° C with the following sampling intervals:

0, 3, 6, 9 , 12, 18 y 24 months and a 6 months accelerated study at 30 º C with sampling intervals of 1, 2, 3 y 6 months. Stability indicating parameters were evaluated during the intermediate intervals. At the end of the study (24 months) all the quality control tests defined in the product specification were evaluated. **Results and Discussion**: All vaccine batches meet the criteria defined in their quality specifications. No variation in Appearance was observed for both types of stability studies. pH, protein concentration and adsorption remained within the acceptable limits. Immunogenicity assays yielded for responding animal titres higher than the cut-off values defined for both serogroups. Likewise, bactericidal titres were above 1/4 dilution, a value considered as protective for Meningococcal vaccines. **Conclusions**: A-W_{135} Meningococcal vaccine based on OMV is stable during 24 months at 2-8 ºC.

20. NEW PROCESS EVALUATION AT LARGE SCALE TO OBTAIN CAPSULAR POLYSACCHARIDE PURIFIED FROM NEISSERIA MENINGITIDES SEROGROUP W_{135}

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Introduction: Although two centuries have passed since Vieusseux described epidemic meningococcal disease, *Neisseria meningitidis (Nm)* remains a leading cause of meningitis and sepsis. There are substantial cyclical fluctuations in meningococcal disease incidence and the occurrence of outbreaks and epidemics. Thus, efforts to control the disease have focused on vaccination. Purified Capsular polysaccharides (PCP) are used in the production of vaccines against these bacterial. W_{135} is one of the five disease causing *Nm* serogroup (A, B, C, W_{135} and Y) and has been of increased interest after an outbreak among Hajj pilgrims in 2000 and an epidemic in Burkina Faso in 2002. The purpose of this work was to obtain PCP from *Nm* serogroup W_{135} by a new process at large scale. **Materials and Methods:** The new process was compared with the current procedure, using the same strain but different media composition and a purification procedure without phenol and ultracentrifugation steps. **Results:** The new process time was the halve, and the yield increased more than 7,7 folds, with great impact in cost reduction and environmental. **Conclusion:** The PCP obtained fulfills the WHO requirements and was used in a Men ACYW vaccine with successful results in Clinical trial phase I.

21. STABILITY STUDIES FOR POLYSACCHARIDES AND FINAL LOTS OF A TRIVALENT *NEISSERIA MENINGITIDIS* SEROGROUPS A, C AND W_{135} VACCINE

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Introduction: Polysaccharides both used as pharmaceutical ingredients and as components of vaccine formulations should be tested to assign them an expiry date. For that, physico-chemical, microbiological and biological properties must be evaluated under stability schemes in final containers according to the WHO requirements for these products. **Aim** The aim of this study was to evaluate the stability of polysaccharides components and the vaccine against Neisseria meningitidis serogroups A, C and W_{135}. **Materials and Methods**: A shelf life study at -20 °C, an accelerated one at 2-8 °C and an under stress study at 37 °C (at 25 °C for vaccine) were performed as recommended by CECMED. Stability indicating parameters were evaluated during the intermediate intervals. At the end of each study all the quality control tests defined in each product specification were evaluated. **Results**: In general the products met the criteria defined in their quality specifications. Significant differences ($p<0.05$) were observed for residual Humidity for the 3 serogroups and the content of Polysaccharide W. The Molecular integrity of Polysaccharide A was affected at 37 °C with no change for Polysaccharides C and W. Under accelerated

conditions the Humidity percent was affected for the 3 Polysaccharides. For the vaccine, the results met the specified criteria for 3 months at -20 °C, with significant changes in Humidity at 2-8 and 25 °C. **Conclusions**: Shelf life of the vaccine was set up in 3 months (at -20 °C) Polysaccharides should be re-tested at 36 months (A and C) and 24 months (W_{135}).

22. CLINICAL TRIALS AUDIT TO THE *NEISSERIA MENINGITIDIS* A, C, W POLYSACCHARIDE VACCINE FROM FINLAY INSTITUTE

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Introduction: Quality assurance includes the audit activity as a tool for detecting non conformities generating corrective and preventive actions in order to improve the GMP implementation and the Quality System standard. An early implementation of the GMP requirements at the clinical trials stage will favor the Certification and the Licensing processes. So, minimizing the GMP non conformities during the development of the product to be immunized in humans, it will be possible to assure Efficacy and Safety and a reliable application of Good Clinical Practices. **Aims**: The aim of this work was to carry out for first time an audit process to the Clinical Trial for the Polysaccharide ACW vaccine. **Methods:** Clinical Trials regulations from CECMED and ANVISA were consulted. The elaboration of documents for the audit process and the selection of the team were made according to the Finlay SOP. Audit techniques such as document sampling, deep point and in situ verification to demonstrate the sample traceability and the quality of the study were used. Checking Lists were prepared to detect the non conformities. **Results:** After the audit performed to different areas from Finlay institute and the UCI hospital, a total of 11 Non conformities and 25 Recommendations were pointed out. All of them were evaluated and solved by the Finlay Institute Quality System. **Conclusions:** It was demonstrated the usefulness of the Clinical Trials Audit as a tool for assuring the fulfillment of GMP and GCP requirements of the ACW Polysaccharide vaccine.

23. PURIFICATION OF LIPOPOLYSACCHARIDES FROM *N. MENINGITIDIS* FOR USE AS REFERENCE MATERIAL FOR ITS QUANTIFICATION IN VACCINES

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Introduction: The lipopolysaccharide (LPS) is among the main constituents of the outer membrane of meningococcus. Finlay Institute is working on vaccines obtained from outer membrane vesicles (OMV) of *Neisseria meningitidis*. Is possible to make the determination of LPS by KDO molecule measuring for serogroup B, however, for other serogroups is not described the relationship between KDO molecules and the structure of the LPS. LPS from these other serogroups are necessary as reference material for its quantification in vaccines. **Aims:** The objective of this work was to purify the LPS of serogroups A, W_{135}, and X of *N. meningitidis* that may be used as reference material in the quality control of the OMV of vaccines of *N. meningitidis*. **Materials and methods:** The purification was carried out using the method of hot-phenol, follow by ethanol precipitation and rotoevaporation to dryness. The purity of LPS was determined by SDS-PAGE electrophoresis and integrity was analyzed by size exclusion chromatography. It were quantified the amounts of KDO by TBA method and were determined the specific immunotype of the three LPS. **Results and discussion:** Highly purified LPS were obtained, and their integrity were proved. The LPS from serogroups W_{135} and X were identified as immunotype L3,7,9

while serogroup A as L11. **Conclusions:** Highly purified LPS from serogroups A, W_{135} and X of *N. meningitidis* were obtained for the quality control of OMV productions for these serogroups.

24. PRODUCTION OF FREE PORCINE COMPONENTS *NEISSERIA MENINGITIDIS* REFERENCE SEED LOTS

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Introduction: During the manufacturing of biological products derived from microorganisms, stable and well-characterized cells to be used as production source should be provided. In this sense, it is essential to count on culture media guarantying an optimal growth of the microorganisms, as well as the presence of the relevant antigens. At the moment, Finlay Institute works in obtaining a vaccine against *Neisseria meningitidis* serogroups A, C, Y and W135 free of components of porcine origin. *Neisseria meningitidis* is considered a demanding microorganism taking into consideration it only grows in enriched means, within tight limits of temperature and pH. **Aim:** To obtain Reference Seed Lots of *Neisseria meningitidis* serogroups A, C, Y and W135 by using culture media free of components of porcine origin. **Results:** Seed Lots from each serogroup were obtained. The characterization studies demonstrated a suitable viability and the identity and purity remain unchangeable. New documentation was established, including Lot Master Files, Standard Operational procedures, lot records, quality control specifications and assay certificates. **Conclusions:** We developed new reference Seed Lots of *N. meningitidis* serogroups A, C, Y and W135 as a first stage in the production of free porcine component vaccine batches.

25. STABILITY OF WORKING SEED LOT OF *NEISSERIA MENINGITIDIS* SEROGROUP X GROWN IN CULTURE MEDIA OF NON-ANIMAL ORIGIN AND PRESERVED BY FREEZING

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Introduction. *Neisseria meningitidis* is an organism that has special nutritional requirements for growth, so strains have grown from simple culture media without animal ingredients, and stable to freezing, would be of great impact on vaccine production .**Aims.** To evaluate the stability of the strain Bufa 2/97 *Neisseria meningitidis* serogroup X cryopreserved in 15% glycerol. **Methods.** Was evaluated the growth in tryptone soy, non-animal and effectiveness MLNM culture medium to increase of cell growth, viability, and polysaccharide yields. The growth kinetics was followed by turbidimetry at intervals of 1 hour. **Results**. The behavior of the strain in the tested media was similar to others traditionally used for the growth of this organism without morphological changes or structural. The kinetics of growth were exponential from the beginning of culture, no significant differences between the optical densities of the cultures were observed, and comparable results in terms of bacterial growth and increase of antigenic expressions in evaluated times were obtained. **Conclusion.** Strain showed to be stable to freezing maintaining the viability in the same logarithmic order of 10^8 CFU / mL, without the presence of others contaminating and the control techniques used, including the proton nuclear magnetic resonance, showed that the polysaccharide is obtained with the quality specifications in agreement settled down in our Institute for this products.

26. *NEISSERIA MENINGITIDIS* SEROGROUP X: ANALYTICAL CHALLENGES AND ALTERNATIVES FOR EVALUATION OF POLYSACCHARIDE CONTENT BY QUANTITATIVE NUCLEAR MAGNETIC RESONANCE

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Introduction: ***N. meningitidis*** constitutes the main responsible of meningococcal disease in infants. Serogroups A, B, C, W135, Y, and X have the higher incidence in young children and teenagers. Recently, serogroup X (PsX) has been found to be responsible of different outbreaks of meningococcal diseases, mainly in "Meningitis Belt" of Africa. In the last decade, the application of quantitative nuclear magnetic resonance had an increasing impact on the pharmaceutical industry. **Aims.** In this study a couple methods by quantitative phosphorous NMR (qPNMR) and proton NMR (qHNMR) are reported for the PsX quantification. **Methods.** Sample of PsX was supplied by the Department of Technological Development from the Finlay Institute. Three set of samples were prepared dissolving different quantities (5 – 22 mg) of capsular polysaccharide in 0.6 mL of deuterium oxide. For phosphorous spectra, one capillary with di-sodium hydrogen phosphate (Na_2HPO_4) as reference was inserted into the 5 mm NMR tube. For proton spectra a solution of deuterium oxide with 3-(trimetylsilyl) 2,2,3,3-tetra-deuteropropionic acid sodium salt (TPS-d_4) (1 mg/mL) was employed to dissolve the sample. NMR analyses were carried out on a Bruker Avance DPX 250-MHz instrument operating with a 5 mm QNP z-axis gradient probe. An inversion – recovery experiments were run for the relaxation times calculation. **Results.** The evaluated assays have been shown to be linears (proportional bias less than 1 %), accurates and precises for intermediate precision conditions (relative standard deviation less than 2.5 % for analyst-to-analyst variations) **Conclusion.** Both optimized experiments allow the quantitative analysis of the PsX polysaccharide.

27. CONJUGATION OF CAPSULAR POLYSACCHARIDES FROM *NEISSERIA MENINGITIDIS* SEROGROUP X TO TETANUS TOXOID

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Introduction: *N. meningitidis* is a leading cause of bacterial meningitis and sepsis throughout the world. There are thirteen different serogroups of this bacterium, which have been identified on the basis of the immunological specificity of capsular polysaccharides (PsC). Six of these serogroups (A, B, C, W135, Y and X) are associated with the majority of meningococcal diseases. Recently, outbreak and increases of incidence of serogroup X have been reported in Meningitis Belt, in Africa. Successfully, conjugate vaccine has demonstrated be more efficient to prevent meningitis diseases than polysaccharides vaccine, however nowadays, licensure conjugate vaccine against this microorganism does not covering this serogroup. For these reasons, to prepare a conjugate vaccine with capsular polysaccharide form serogroup X that can be including in a multivalent conjugate vaccine, would provide a major coverage vaccine. Center of Biomolecular Chemistry and Finlay Institute have a research project to obtain a

conjugated vaccine against serogroups A, C, W135, Y and X of *N. meningitidis*. **Aims:** Here, we report the obtaining of conjugate from *N. meningitidis* serogroup X to Tetanus Toxoid. **Methods:** The work methodology consisted: i. Fragmentation of PsC, ii. Activation, iv. Conjugation. The final products of these reactions were characterized by chemistry-physics techniques. The conjugates were immunized in Balb/C mice and the immunological response was evaluated by immunoenzymatic assays. **Results**, Good recovers in fragmentation and activation reactions were achieved. The conjugates had slow percent of free protein. The antibody titles elicited by the conjugates were high against PsC. **Conclusion.** The conjugates obtained using the proposed methodologies were immunogenic.

28. VACCINE POTENTIAL OF OUTER MEMBRANE VESICLES FROM *NEISSERIA MENINGITIDIS* SEROGROUP X

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Introduction: Meningococcal disease is caused mainly by *N. meningitidis* serogroups A, B, C, Y and W_{135}. However, numerous cases of meningitis caused by *N. meningitidis* serogroup X have recently been reported in several African`s countries. Currently, there are no licensed vaccines against this serogroup. Finlay Institute has experience in the production of outer membrane vesicle (OMV) vaccine from *N. meningitidis*. The aim of this work is to obtain, characterize and evaluate an OMV candidate vaccine from serogroup X (OMVx). **Methods**: Three experimental lots of OMVx were obtained by deoxycholate extraction method from *N. meningitidis* serogroup X BuFa 2/97 strain. Physico-chemical characterization was carried out to determine the size, morphology and the main antigens in vesicles. OMVx were adsorbed to aluminum hydroxide (OMVx/AL) and were administered alone or in combination with capsular polysaccharide X (PsX) or polysaccharide A (PsA). Antigen specific IgG responses induced by these formulations to polysaccharides or OMVx were evaluated by ELISA, and serum bactericidal assay (SBA). **Results**: OMVx size's was between 90 and 120 nm. OpcA, RmpM and 70 kDa protein antigens were identified. OMVx/AL elicited high anti-OMVx antibodies responses with bactericidal activity. Co-administration of PsX or PsA with OMVx/AL enhanced both antibodies response to polysaccharides and bactericidal activity against serogroup A and X. However, no immunogenicity was recorded from formulations of polysaccharide administered with AL adjuvant only. **Conclusion**: OMV obtained from *N meningitis* serogroup X were immunogenic and were able to potentiate immune response when PsX or PsA were co administered. These results suggest a switch from thymus (T) -independent to T-dependent response of polysaccharides. Novel formulations will be designed to protect against meningococcal disease in Africa.

29. AND CHARACTERIZATION OF CONJUGATES FROM *STREPTOCOCCUS PNEUMONIAE* SEROTYPES 7F, 9V AND 19A TO DIFFERENT CARRIER PROTEINS.

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Introduction: *Streptococcus pneumoniae* is the most common cause of acquired pneumonia, meningitis, and bacteremia in children and adults. In Cuba, this bacterium is responsible of more than 25 000 cases

of pneumonia and 300 cases of meningitis per year, mainly in children under 5 years old. Multivalent pneumococcal conjugate vaccine is, therefore, a high priority for Public Health. Our laboratory has development a heptavalent conjugated vaccine against this bacterium in order to protect the Cuban children. Among the prevalent serotypes associated with infections pneumococcal diseases worldwide are 7F, 9V and 19A but these are not included in our first vaccine candidate. **Aims**: Study the conjugation procedure of these serotypes to different carrier proteins to increasing the coverage of vaccination. **Methods and Results:** We have developed a process that includes activation of polysaccharide by peryodic oxidation and conjugation to different carrier protein using reductive amination. The obtained conjugates were characterized by HPLC, colorimetric method to determine protein polysaccharide ratio (w:w) and inmunoenzimatic assay. The immunologic evaluation in mice shows that the conjugates obtained using this methodology are antigenic and evokes specific IgG anti-polysaccharides antibodies. **Conclusion:** the conjugates obtained using this methodology, are antigenic and immunogenic in mice.

30. ADJUVATION OF CONJUGATES FROM *STREPTOCOCCUS PNEUMONIAE* SEROTYPE 1 AND 14 TO TETANUS TOXOID IN ALUMINIUM COMPOUNDS. IMPACT IN THE IMMUNE RESPONSE

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Introduction. The adjuvation in aluminum phosphate is a technological tool used to enhance the immunogenic response in vaccines. **Aims.** In this work we define the conditions that allow absorbing more than 80% of antigen by the evaluation of pH, time and concentration of protein using tetanus toxoid as a model. **Methods.** The mathematical model that describes the process and the main chemical and physical variables were determined. In addition, we evaluated the effect of the ionic strength and ethylene glycol on the adsorptive capacity. Finally we applied the methodology in the adjuvation of conjugates from *Streptococcus pneumoniae* serotype 1 and 14 to tetanus toxoid and evaluated the immune impact of the adjuvant in mice. **Results.** The results show that an adsorption greater than 80% was obtained using pH 6 and 2 hour of reaction. The process can be described by the Langmuir isotherm, showing hight affinity of the carrier and the conjugates for the surface of the aluminum phosphate. The immunologic evaluation shows that the conjugate 1-TT adjuvated induces antibodies IgG with higher avidity and promote affinity maturation compare with non-adjuvated, but the adjuvant doesn't improve the levels of antibodies IgM or IgG anti-PS in serum or mucosa. For conjugate 14-TT, adjuvant induces higher levels of IgG anti-PS after second dose, marked increment of IgG1 in serum and IgG in respiratory mucosa compare with the same conjugate without adjuvant. **Conclusion.** In conclusion the adjuvation procedure guaranties the adsorption in AlPO4 and improves the immunogenicity of monovalent conjugates 1-TT and 14-TT.

31. GROUP B MENINGOCOCCAL VACCINE CANDIDACY OF HAEMOGLOBIN RECEPTORS BASED ON DISTRIBUTION AND PHASE VARIABLE STATUS.

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Introduction. Owing to the poor immunogenicity of the meningococcal group B (MenB) capsule and diversity of subcapsular antigens, no universal MenB vaccine currently exists. The surface exposure of the haemoglobin receptors HmbR and HpuA, and their apparent interaction with the immune system, makes them potential candidates in the pursuit of vaccine antigens to broaden MenB strain coverage. Variable distribution and phase variability of these antigens, however, raises possible doubts over their usefulness. **Aims.** To investigate the distribution and '*in vivo*' phase variable status of hmbR and hpuA among eighty representative invasive MenB disease cases in England, Wales and Northern Ireland. **Methods.** Polymerase chain reaction, sequence analysis and GeneScan fragment analysis of *hmbR* and *hpuA* in invasive disease isolates and their corresponding clinical specimens. Phenotypic analysis of the ability of invasive disease isolates to grow using haemoglobin as the sole iron source. **Results and conclusion.** Consistent with the requirement of iron acquisition for invasive meningococcal disease, at least one haemoglobin receptor gene was present and predicted to be expressed among the majority (approximately 76%) of cases. Broader redundancy among iron acquisition mechanisms, specifically in the form of transferrin binding protein (Tbp), was likely to account for the remaining cases. A vaccine formulation encompassing these and complementary iron acquisition systems is worthy of further investigation to broaden MenB strain coverage. The approach used in this study can be applied to other phase variable genes and will help with understanding the contributions of phase variation to invasive meningococcal disease.

32. COMPARISON OF CYTOKINE GENE POLYMORPHISMS AMONG GREEK PATIENTS WITH INVASIVE MENINGOCOCCAL DISEASE OR VIRAL MENINGITIS

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Introduction: Genetic predispositions for low pro-inflammatory and high anti-inflammatory responses were observed among first-degree relatives of patients who died of invasive meningococcal disease (IMD). Among ethnic groups in which IMD is more prevalent and/or more severe (*e.g.* Aboriginal Australians) there is a predominance of cytokine gene profiles predicted to result in high pro-inflammatory responses and low anti-inflammatory responses. **Aim:** As high levels of pro-inflammatory cytokines are implicated in severity of IMD and viral meningitis (VM), we assessed single nucleotide polymorphisms (SNPs) of pro- and anti-inflammatory cytokine genes among patients with VM or IMD. **Methods:** Patient DNA samples were prepared by the National Meningitis Reference Laboratory in Athens: IMD=98; VM=53. Results were compared with data published for healthy Greek controls.SNPs were assessed by real-time PCR: *IL6* G-174C, *IL1B* C-511T, *IL1RN* T+2018C, *IL10* G-1082A, *IL8* A-251T and *TNF* G-308A, differences compared by Fisher's exact test. **Results: G**enotypes for high IL-6 responses were predominant among IMD (51%, p=0.0008) and VM (74.5%, p< 0.0001) compared with controls (31%). Genotypes associated with high TNF-α responses were 5% among controls, lower for IMD (1.1%, p=0.0014) and VM (0%, p=0.052). There was no difference for IL-8 SNPs between controls and IMD (p=0.162) but significant for VM (p=0.0025). IL-6 (p=0.024) and IL-8 (p = 0.00004) SNPs differed between IMD and VM. **Conclusions:** Reports on associations between IL-8 SNPs and cytokine responses differ. Because of its role in neutrophil attraction, differences in frequencies of the IL-8 SNP for IMD and VM require further investigation.

33. A NOVEL FACTOR H-FC CHIMERIC IMMUNOTHERAPEUTIC MOLECULE AGAINST *NEISSERIA GONORRHOEAE*

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Neisseria gonorrhoeae has become resistant to almost every conventional antibiotic, thus attaining 'superbug' status. There is an urgent need to develop novel therapies against this pathogen. The gonococcus binds human factor H (fH), an inhibitor of the alternative complement pathway, through fH domains 18-20. We have shown previously that a chimeric molecule comprising fH domains 18-20 fused to murine IgG2a Fc (fH18-20/Fc) bound to gonococci and mediated complement-dependent killing. However, the use of native (unmodified) fH domains 18-20 in such a molecule could result in binding to host cells, complement activation and subsequent tissue damage. Mutations in fH domains 19 and 20 that alter binding of fH to polyanions and/or C3b occur in persons with atypical hemolytic uremic syndrome (aHUS). We created four fH18-20/Fc mutant proteins that incorporated individual aHUS mutations and evaluated them for binding to and complement-dependent killing of gonococci. Recombinant fH 19-20 molecules that displayed one of these mutations, D1119G, was incapable of inhibiting native fH-mediated protection of anti-CD59-treated human RBCs from complement-mediated lysis (Ferreira et al, J Immunol, 2009, 182(11):7009). The D1119G mutant (fH18-20(D1119G)/Fc) showed binding and killing of gonococci comparable to the wild-type fH18-20/Fc. In contrast to the wild-type fH18-20/Fc, fH18-20(D1119G)/Fc did not cause hemolysis of anti-CD59-treated human RBCs and showed less C3 deposition on retinal pigment epithelial cells compared to fH18-20/Fc wt. These data identify fH18-20 (D1119G)/Fc as a promising anti-gonococcal immunotherapeutic. Ongoing in vitro studies show less activation of complement on host cells than fH18-20/Fc wt, indicating a favorable safety profile. Several pathogens bind the C-terminus of fH and the utility of fH 18-20(D1119G)/Fc against these microbes merits investigation.

34. ANTIMICROBIAL RESISTANCE PATTERNS IN CUBAN GONOCOCCAL STRAINS

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Introduction. Gonococcal emergence of resistance to antimicrobial agents has made the treatment of gonorrhea expensive and prolonged, as well as unpredictable. Antimicrobial resistance against almost all the antimicrobial agents is spreading therefore surveillance was considered mandatory to develop strategy and policy for proper therapy. **Aims**. To summarize and compare the resistance patterns of consecutive isolates of *N. gonorrhoeae* in Cuba for three years (2010-2012) by E-test strip diffusion methods. **Results**. Of 60 strains investigated in 2012, 30 strains in 2011 and 2010, the susceptibility to penicillin decreased from 26.7% to 6.7%. Similar results were observed for tetracycline with a reduction to 11.7% in the last year. The high resistance to ciprofloxacin was maintained for all the period (66.7%; 48.5% and 61.7%) and 100% of all strains were susceptible ceftriaxone and spectinomycin. The resistance pattern with 69.2% to ciprofloxacin, 40% to tetracycline and 34% to penicillin of the strains all over these years was very high. **Conclusions**. Fortunately a similar behavior of antimicrobial resistance patterns was observed during 3 years only with a decrease of susceptible strains, nevertheless, we have to keep alert about the high percentage of fluoroquinolone-resistance strains as well as the resistance percentage increase of penicillin and tetracycline similar to those observed in 90´s. Despite the moderate number of strains tested, these results have an effect for antimicrobial management of gonorrhea in Cuba.

35. THE UTILITY OF PCR IN DIAGNOSTIC OF UNSPECIFIED AND TREATED BACTERIAL MENINGITIS IN CUBA

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Introduction. Bacterial meningitis is often strikes suddenly and can either result in death with serial neurological dysfunctions. The illness surveillance, leadership by the Ministry of Public Health, constitutes one of the major activities of regional laboratories in Cuba. Conventional bacteriological methods uses in these laboratories frequently fail to identify an agent, as a result of administration of antibiotics or delayed lumbar punctures. **Aims**. To determinate the major aetiologic sources of unspecified and post-treated bacterial meningitis by polymerase chain reaction (PCR)-base identification of *Neisseria meningitidis* (crg), *Streptococcus pneumonia* (ply) and *Haemophilus inlfuenzae* type b (bexA). **Results**. One hundred and eighty four cerebrospinal fluid samples received in to the National Reference Laboratory from 2009 to 2012 were tested. The multiplex PCR detected *N. meningitidis* in 1.6%, *S. pneumoniae* in 8.1% and 1.0% as *Haemophilus inlfuenzae* type b. In addition, *N. meningitidis* positive samples were classified two as serogroup C and one as B by PCR. **Conclusions**. Despite the low positive percentage, bacterial DNA detection was found to be valuable adjunct to enhance bacterial meningitis surveillance when the yield of specimens by culture is reduced. The implementation of PCR assays as a support diagnostic for the Public Health Laboratories surveillance network is perceived to be a significant advance in the investigation of bacterial meningitis in Cuba.

36. EPIDEMIOLOGICAL MARKERS AND ANTIMICROBIAL SUSCEPTIBILITY OF INVASIVE STRAINS. CUBA, 2002-2011

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Introduction: *Neisseria meningitidis* is one of the main causative agents of bacterial meningitis and sepsis. The study of epidemiological markers and antimicrobial susceptibilities of the circulating strains constitute the main activities of meningococcal disease surveillance. **Aims:** To investigate epidemiological markers and the antimicrobial susceptibility patterns of invasive isolates of *N. meningitidis* in Cuba for ten years (2002-2011). **Methods:** A transversal descriptive study was conducted to characterize invasive meningococci isolations received at the National Reference Laboratory of Pathogenic Neisseria during 10 years. A total of 69 viable meningococcal strains were studied. Conventional laboratory methods were used to identify the specie and serogroups; PCR technique was used when the latter was doubtful. Phenotypic characterization was carried out using ELISA of whole cells with monoclonal antibodies, while antimicrobial susceptibility was determined by the E-test method. **Results:** Strains of the serogroup B (98.6%) was predominant, with only one strain belonging to the serogroup C (1.4%); strains of the serotype 4 were the most frequent (55.1%), followed by non-typable and serotype 2a (15.9% and 8.7%), respectively. The more frequent subtypes were P1.15 (42.0%), P1.19 (20.3%) and non subtypable strains (10.1%). Of the serogroup B strains, phenotype B:4:P1.15 was predominant (26.1%), and phenotype C:2a:P1.5 was detected for the first time in Cuba. Susceptible meningococci strains were predominant: ceftriaxone (100.0%), rifampicin (98.2%), chloramphenicol (92.8%), ciprofloxacin (89.3%), and penicillin (87.5%), except for cotrimozaxol, to which however the 92.8% of isolations were resistant. **Conclusions**: Our data provide valuable epidemiological information for a better understanding and control of meningococcal disease in Cuba.

37. INTRATHECAL SYNTHESIS OF COMPLEMENT SYSTEM PROTEINS IN PATIENTS WITH EOSINOFILIC MENINGITIS DUE TO *ANGIOSTRONGYLUS CANTONENSI*

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Background: *Angiostrongylus cantonensis*, is the most common cause of eosinophilic meningitis in many countries including Cuba. Complement is a central component of the innate immune system, it serves as a natural adjuvant, enhancing and directing the adaptive immune response. Cerebrospinal fluid (CSF)/serum quotient graphs or reibergrams have been used previously to determine local synthesis in brain of immunoglobulins. Aim: To determine whether the complement system may be activated by the classical or lectin pathway using the respective reibergrams in patients with eosinophilic meningitis and whether this system might be involved in natural removal of third-stage larvae when the parasite reaches the central nervous system (CNS). Material and methods: CSF and serum samples were obtained from 20 patients with eosinophilic meningitis caused by A. cantonensis. C3c and C4 levels in serum and CSF were quantified by using a radial immunodifusion technique. Mannan binding lectin (MBL) was quantified by inhouse time-resolved immunofluorometric assays. Results: Results were plotted on a C3c, C4 and MBL CSF/serum quotient diagram. Twelve patients showed intrathecal synthesis of C4, thirteen, intrathecal synthesis of C3c and four patients shown MBL intrathecal synthesis. Antibody-dependent complement cytotoxicity should be considered as a possible mechanism that destroys third-stage larvae of this helminth in CSF. Conclusions: Under all conditions of the blood-CSF barrier, the Reibergram can identify the occurrence of C3c, C4 and MBL intrathecal synthesis. It can quantify the complement system proteins fraction which are locally produced in the CNS and differentiate from those proteins that may have entered CSF from blood.

38. MASP2 INTRATHECAL SYNTHESIS _IN EOSINOPHILIC MENINGITIS DUE TO *ANGIOSTRONGYLUS CANTONENSIS*

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Introduction: *Angiostrongylus cantonensis* meningitis was first reported in Cuba and in the Western hemisphere 30 years ago as an emergent disease. **Aim**. Determine CSF soluble MASP2 (mannan-binding-lectin associated serine protease type 2) intrathecal synthesis in patients suffering from eosinophilic meningitis due to *Angiostrongylus cantonensis*. **Method**. MASP2 in CSF and serum samples was quantified by ELISA. 20 controls and 8 patients were studied. IgG and albumin were quantified by immunodiffusion in all samples. **Results**. CSF MASP2 levels are significantly increased in comparison with the expected values according to the normal blood-CSF transference. 50% of the patients showed intrathecal synthesis of MASP2 and its synthesis percentage varied between 20 to 80% of the total CSF MASP2 content. **Conclusion**: It is possible to find intrathecal synthesis of MASP2 in eosinophilic meningitis due to *Angiostrongylus cantonensis.*

39. MASP2: DYNAMICS AND INTRATHECAL SYNTHESIS.

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Introduction. There is an increasing interest to look for the possible participation in lectin pathway components in the immune response in CNS. **Aims.** To find out the role of MASP2 in cerebrospinal fluid and its dynamics through the blood brain barrier **Methods.** MASP2 was assayed in 20 control samples of CSF and serum with an ELISA, coated with anti MASP2 antibodies. Routine parameters such as albumin-, immunoglobulin- CSF/serum quotients, oligoclonal IgG and cell count were used to characterize the patient groups. Groups comprised firstly, control patients without organic brain disease with normal CSF and normal barrier function and secondly, patients without inflammatory diseases but with increased QAlb, i.e. with a blood CSF barrier dysfunction.**Results.**MASP2 concentration in CSF was at least two-fold higher than expected for a molecular-size dependent passage from blood. Secondly, in QMASP2 vs QAlb regression line 9/20 cases showed an intrathecal fraction. The higher inter-individual variation of MASP2 concentrations in CSF of the control group (CV = 55%) compared to the MASP2 concentrations in serum (CV = 40%) indicate that MASP2 is mostly a blood protein. 4) The absolute MASP2 concentration in CSF increases with increasing QAlb. **Conclusions.**MASP2 in CSF is predominantly blood-derived. The evaluation of this protein requires the interpretation of its absolute concentrations in CSF as a function of the albumin quotient, QAlb.

40. IMMUNOLOGICAL RESPONSE IDENTIFIED IN CEREBROSPINAL FLUID IN PATIENTS WITH NEURODEGENERATIVE DISEASES

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Our scientific interest in neurodegenerative diseases is focused on neuroimmunological aspects in prion diseases and other neurodegenerative dementia such as Alzheimer's disease. Therefore, we anaylsed a apanel of prto-and anti-inflammatory cytokines in CSF in patients with various neurodegenerative diseases and present the data below. In a first approach anti-inflammatory cytokines IL-4 and IL-10 were investigated in cerebrospinal fluid samples. In total 61 patients were analyzed. The group was composed of patients with CJD (n = 20), patients with other forms of dementia (n = 10), patients with motoneuron disease (n = 6), patients with normal pressure hydrocephalus (n = 5) and control subjects (n = 20). Interleukin 10 levels were significantly elevated in the cerebrospinal fluid of CJD patients (median, 9.8 pg/mL). The elevation was significant to other dementia (median, 7.9 pg/mL, P<.05), motoneuron disease (median, 7.9 pg/mL, P<.05), normal pressure hydrocephalus (median, 7.0 pg/mL, P<.05), and controls (median, 1.3 pg/mL, P<.001). Levels of interleukin 4 were significantly elevated in cerebrospinal fluid of patients with CJD (median, 26.4 pg/mL) compared with control subjects (median, 6.2 pg/mL, P<.001) and patients with a motoneuron disease (median, 10.5 pg/mL, P<.001). We could also detect elevated levels of IL-4 and IL-10 in the CSF of patients with dementia other than CJD. The group of patients with dementia was mainly composed of patients with Alzheimer's disease and interestingly elevated levels were observed in this subgroup as well. (Figure 2, from Stoeck et al. Arch Neurol (62), 2005 p. 1591-1594.

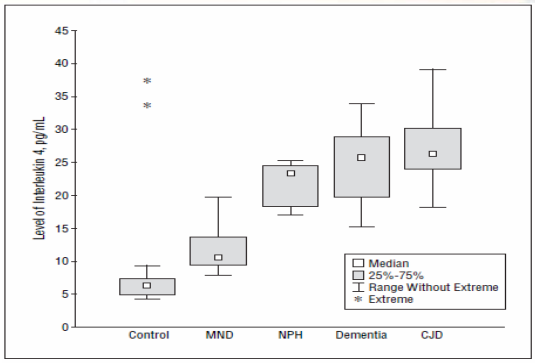

Figure 1. Level of interleukin 4 in cerebrospinal fluid in patients with Creutzfeldt-Jakob disease (CJD), various other neurological diseases, and controls. MND indicates motoneuron disease; NPH, normal pressure hydrocephalus.

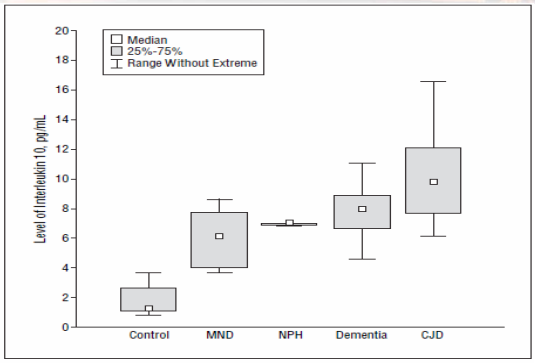

Figure 2. Level of interleukin 10 in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease (CJD), various other neurological diseases, and controls. MND indicates motoneuron disease; NPH, normal pressure hydrocephalus.

In a next step I investigated pro- and anti-inflammatory cytokines in CSF of CJD patients (n=23) as well as from patients inflammatory CNS diseases (n=76), patients with dementia, including AD (n=31), patients with epileptic seizures (n= 18) and controls (n=111). Concentrations of pro- and anti-inflammatory cytokines IL-1h, IL-6, IL-8, IL-12, TNF-a and TGF-β 2 were determined using ELISA. Significant changes were found for IL-8 and TGF-β 2. IL-8 levels were elevated in the CSF of CJD patients (Figure 3 A). There was a marked difference between CJD and other forms of dementia (p =0.08), specifically between CJD and Alzheimer's disease, which is the most important differential diagnosis (p =0.07). (Figure 3 B). In contrast, TGF-β 2 was significantly decreased in CSF of CJD compared to all groups. IL-1β, IL-12 and TNF-a could not be detected in CSF or in case of IL-6 in only low concentrations without significant difference. **Figure 3**: from Stoeck et al. / Journal of Neuroimmunology 172 (2006) 175– 181.