KNA-1: MUCOSAL APPROACHES IN NEISSERIA VACCINOLOGY


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Background and Aims. Meningococcal B strains accounts for some 72% and 28% of meningococcal diseases in infants and toddlers in Europe and the USA, respectively. Nevertheless, meningococcal diseases are rare in Cuba owing to the wide spread program on anti-meningococcal vaccination in the country. Finlay Institute is one of the pioneering organizations in Neisseria Vaccinology mainly by its contribution to N. meningitidis serogroup B outer membrane-based bivalent vaccine, VA-MENGOC-BC®. This vaccine was given intramuscularly in more than 60 million doses corresponding 10.7 millions of them to Cuban young adults, children, and infants. However, most dangerous or commensally Neisseria strains enter and establish in the mucosa, where the secretory (S) IgA is the main specific guardian and is mainly induced by mucosal routes. However, few mucosal vaccines exist principally due to the absent of mucosal adjuvants. We develop a Finlay Adjuvant (AF) platform based in outer membrane vesicles (Proteoliposome, PL) and its derivate Cochleate (Co). AFPL1 derived from serogroup B N. meningitidis is a potent Th1/CTL driving parenteral adjuvant. AFCo1 is a potent mucosal adjuvant. Therefore, we sought to go deeper in the possible mucosal cross recognition between N. meningitidis serogroups and Neisseria species and explore a concurrent mucosal and parenteral immunization strategy (SinTimVaS) in order to develop suitable mucosal vaccines. Methods. Experiments were conducted in Balb/c or C57Bl6 mice with mucosal and systemic immunization using AFCo1 and AFPL1. Human sera and saliva were also analyzed for cross cognition. Results. Mucosal cross recognition at SIgA level in human saliva between N. meningitidis serogroups B, A, C, Y, and W135 were observed. This SIgA cross recognition response was also observed between pathogenic (N. meningitidis serogroup B, N. gonorrhoeae) and non-pathogenic strains (N. flava, N. lactamica). The possible influence of meningococcal vaccination against Gonorrhea was also explored. A proprietary Single Time Vaccination Strategy combining simultaneous mucosal and parenteral doses was developed. Conclusions. N. meningitidis and N. gonorrhoeae show significant cross immune response, and mucosal immunization with AFCo1 to obtain immunity against both strains may be a useful strategy to combat both infections in humans. Single Time Vaccination Strategy could be important to increased human vaccination coverage and herd immunity protecting both systemic and mucosal environments.

Symposium I: New Neisserial antigens discovery

KNA-2: PERSPECTIVES IN NEISSERIA VACCINE DEVELOPMENTS

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MenACWY: After the success of the development and introduction of Hib conjugate vaccines, meningococcal C, CY, A and ACWY conjugate development has been undertaken and lead to various applications. The introduction of meningococcal conjugates has been accompanied in a number of countries with catch-up campaigns including children and teenagers. This resulted in the Netherlands in a disappearance of MenC disease in the first year of life without actual immunization of this target population. Ongoing efforts to reduce the burden of MenA disease in Africa aim to follow the success of the European MenC catch-up campaigns. An open question is how long herd effects and reduced transmission patterns after a catch-up campaign will last to allow protection for the non-immunized infants. An alternative approach for the use of meningococcal conjugates implies direct protection of infants, probably supplemented with routine teenager immunization. MenB: The development of MenB vaccines has been successful for the control of outbreaks due to a single or relatively few bacterial clones, although the application of outer membrane vesicle vaccines has mostly limited itself to clonal outbreaks in Cuba, Norway and New Zealand. A hexavalent OMV approach has been attempted, and if pursued bi-, tri- etc valent OMV vaccine approaches may allow the control of hyperendemic waves such as has occurred due to the ST41/44, P1.4 upsurge in Europe in the 1990’s. A preferred scenario however is the development of generic MenB vaccine compositions covering the majority of the globally circulating disease causing clones/strains.
Meningococcal meningitis and sepsis are devastating diseases that can kill children and young adults within hours, regardless of the availability of effective antibiotics. Despite a lot of research efforts, today there are no effective vaccines available for the prevention of *Neisseria meningitidis* serogroup B (MenB). Using a new strategy, named “Reverse Vaccinology”, based on the whole-genome sequencing of a serogroup B strain and its analysis by computer-aided methodologies we have discovered previously unknown surface exposed proteins, able to induce bactericidal antibodies, a property known to correlate with vaccine efficacy in humans. The vaccine antigens selected were prioritized based on their ability to induce broad strain coverage as inferred by bactericidal activity. Three recombinant antigens (two fusions and one single protein) have been expressed in a form suitable for large-scale manufacturing and formulated with adjuvants suitable for human use. The vaccine induces bactericidal antibodies in mice against 78% of a panel of 85 genetically diverse meningococcus strains and the coverage can be increased to 90% using different adjuvants. Human clinical trials in adults and infants demonstrated satisfactory tolerability and immunogenicity. The newly identified antigens have been further characterized by the biochemical, immunological and functional points of view. Functional data suggest important roles in virulence and pathogenesis for three vaccine antigens named fHBP, NadA and GNA2132. fHBP is a unique vaccine antigen since it induces antibodies that have both complement-mediated bacterial killing activity and that inhibit binding of factor H to the bacterial surface increasing the susceptibility of the bacteria to lysis by human complement. NadA has been characterized as a surface-exposed adhesin involved in the interaction with human epithelial cells suggesting that NadA antibodies might also prevent meningococcus colonization. GNA2132 is a surface-exposed lipoprotein able to bind heparin a feature usually associated with increased adherence to host tissues and with enhanced serum resistance. Considering the particular nature of these vaccine antigens, vaccine escape mutants are unlikely to be successful since they have reduced survival and virulence.

**C-2: THE ROLE OF MENINGOCOCCAL SECRETED PROTEINS IN PATHOGENESIS AND PROTECTION**

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Meningococcal secreted proteins (MSPs) play essential roles in host–bacterial interactions and in the pathogenesis of disease. We have previously demonstrated the differential host gene expression in human meningococcal-derived cells, in response to MSPs [1]. Using expression arrays, upregulated expression of several pro-inflammatory and apoptosis-related genes was found to be induced by MSPs. Upreregulated IL-8, IL-6, ICAM-1 and COX-2 transcription and protein expression were confirmed by real-time PCR, ELISA, flow cytometry and/or Western immunoblots. Exposure of cells to MSPs increases resistance to staurosporine-induced apoptosis. More recently, we addressed the hypothesis that MSPs can elicit protective immunity against meningococcal infection. Vaccine 2009. In press
To identify novel vaccine candidates to be included in a vaccine composition against *N. meningitidis* we combine several approaches: 1. Genome mining, 2. Qualitative and quantitative proteomics using two-dimensional gel electrophoresis, dual C-5: VACCINE CANDIDATES AGAINST NEISSERIA MENINGITIDIS IDENTIFIED AT THE CENTER FOR GENETIC ENGINEERING AND BIOTECHNOLOGY

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To identify novel vaccine candidates to be included in a vaccine composition against *N. meningitidis* we combine several approaches: 1. Genome mining, 2. Qualitative and quantitative proteomics using two-dimensional gel electrophoresis, dual C-3: STUDY OF NEISSERIA MENINGITIDIS OMV USING PROTEOMIC TECHNIQUES


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At present one of the efforts devoted to the development of an effective vaccine against *Neisseria meningitidis* serogroup B have been focused in the proteomic studies of outer membrane vesicles (OMV). This work presents the results from a detailed study of the protein composition of OMV from VA-MENGOC-BC® (Finlay Institute, Cuba). The characterization of this protein preparation is a challenge because of it is enriched in lipids and membrane proteins and only a few number of proteins represent about 70% of the total protein mass. Proteins were identified by, combining two-dimensional gel electrophoresis of proteins and peptides and mass spectrometry, and the application of non-gel based approaches starting form the tryptic digest of the OMV preparation and (1) the Selective CApture of PEptides (SCAPE-nHnR), (2) peptide fractionation by reverse phase liquid chromatography at basic pH, and (3) equalization of the peptide mixture by using equalizer peptide libraries before LC-MS/MS analysis. This study resulted in the identification of more than one hundred proteins. Bioinformatics analysis of the identified components allowed the selection of potential antigens for cloning, expression, purification and immunological studies, which were part of a wider project aimed to the development of a new vaccine based on a defined protein composition.

C-4: IMMUNOPROTEOMIC ANALYSIS OF THE IMMUNE RESPONSE TO MENINGOCOCCI

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The development of immunity to meningococcal infection has been studied by an immunoproteomic analysis of sera obtained following two longitudinal studies of colonisation of students during their first year at university. Sera were assessed for bactericidal activity against carriage strains isolated during the study. Acquisition of carriage was associated with the presence of serum bactericidal activity (SBA) against the homologous carriage strain and also heterologous strains. Sera were selected which showed an increase in SBA on acquisition of carriage. Outer membranes of meningococci from the carriage isolates were subjected to 2D-electrophoresis and then to immunoblotting with the selected sera obtained prior to and post colonisation. The acquisition of meningococcal carriage which accompanied raised bactericidal antibody activity was associated with increased immunoreactivity visible on the immunoblots. This development of an immune response was reflected both in a greater number of spots and increased intensity of existing spots. The number, range and relative intensities of immuno reactions to meningococcal proteins varied between students and between strains. Twenty seven discrete immunoreactive spots could be identified. The corresponding spots were excised from gels and subjected to mass spectrometry analysis to identify the associated protein antigens. The list of protein antigens generated not only well established protein antigens (as expected) such as PorA but also new potential vaccine candidates associated with the development of cross reactive immunity to serogroup B meningococcal infection.

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fractionation-PAGE and liquid chromatography/mass spectrometry based on selective capture of peptide (SCAPE), 3. DNA expression library computer. The identified antigens were further selected based on: high conservation and expression among N. meningitidis strains; surface exposition measured by complement deposition and whole cell recognition by FACS; feasibility of expression at high levels in E.coli; passive protection capacity against N. meningitidis infection in the infant rat model and serum bactericidal activity (SBA) in vitro. Finally, seven novel antigens were selected. The amino acid length of the chosen antigens ranges from 77 to 365. The seven deduced amino-acid sequences showed 90-100% identity after analyzing the corresponding genes in a panel 41-50 representative strains. The selected antigens induce protection against homologous and heterologous challenger in the infant rat model. In spite of the bactericidal activity of individual sera, additional increases on SBA and protection capacity were observed when sera against different antigens were mixed. Selection of antigens to be included in the final multicomponent vaccine formulation will be based on the increased functionality of mixed sera. To facilitate product development selected antigens will be expressed as fusion proteins.

C-6: LOS AS PUTATIVE VACCINE ANTIGEN: VARIABILITY OF LPS GENOTYPES AMONG A LARGE COLLECTION OF N. MENINGITIDIS B STRAINS AND IMPACT ON THE DESIGN OF A CROSS-REACTIVE ANTIGEN

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Lipo oligosaccharide (LOS) is a major virulence factor of N. meningitidis (Nm) and allows to discriminate 12 different immunotypes. This antigen was shown to induce protective antibodies in human after exposure to invasive or carried Nm strains. For a long time the diversity of LPS immunotypes among meningococci and the toxicity of its LipidA moiety have been considered as an obstacle for the design on an effective vaccine candidate. Three genetic loci (lgt-1, 2, 3) were reported to encode the glycosylases responsible for biosynthesis of the LOS oligosaccharide chains. In addition, meningococcus is able to introduce variable decorations in the inner-core of its LOS by O-acetylation of the N-acetylglucosamine (GlcNAc) and/or by adding phosphoethanolamine groups (PEA) or glucose on Heptose II (HepII). Genes responsible for the addition of such decorations have been described (lot-3 for addition of O-acetyl, lpt-3 and lpt-6 for addition of PEA, respectively at position 3 and 6 of HepII and lgtG for glucose addition on position 3 of HepII). Taking advantage of this genetic knowledge we analyzed a total of 163 N. meningitidis strains representatives of 6 major clonal complexes responsible for serogroup B disease (ST -32, ST -11, ST -41/44, ST-8, ST-269 and ST-18). The majority of those strains were kindly provided by Dr. D. Caugant (NIPH, Norway), Dr D. Martin, (ESR, New-Zealand) and Dr M. Diggle, (SMPRL, Scotland). This collection was used to predict the diversity of LOS structure. Based on inner core PEA decoration, two major LOS types were shown to be represented over more than 80% of the strains studied. This led us to design two vaccine candidates that have been assessed for their ability to induce cross-reactive antibodies among a panel of 30 strains.

C-7: THE VACCINE CANDIDACY OF MENINGOCOCCAL OPA PROTEINS

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Introduction: Meningococcal disease cannot be comprehensively controlled due to the lack of a vaccine against meningococci expressing the serogroup B capsule. The majority of serogroup B and C disease in the past five decades was caused by a small number of hyperinvasive lineages (the ST-8, ST-11, ST-32 and ST-41/44clonal complexes). During decades of global spread, these clonal complexes remained stably associated with limited combinations of highly immunogenic outer membrane adhesins called opacity (Opa) proteins. Recent in vitro studies imply that these proteins may have immunomodulatory effects on T cells mediated by CEACAM1, however, studies of the effects of Opa proteins on human cellular immunity have focussed largely on gonococcal Opa proteins. The aim of this study was therefore to evaluate the immunogenicity in mice of meningococcal Opa proteins, to assess their potential as vaccine candidates, and to examine their immunomodulatory properties in vitro with human PBMCs. Methods: Fourteen oap genes were cloned, expressed in E. coli, refolded, and purified using affinity chromatography prior to immunization with solubilised recombinant protein adsorbed onto aluminium hydroxide or mixed with Freund's adjuvant.
Responses in pooled murine sera were detected using a Serum Bactericidal Assay (SBA) with meningococci from the same clonal complex as targets. Total serum IgG levels were assessed by whole cell ELISA against the same isolates and the specificity of the antibodies determined by Western immunoblot. The immunomodulatory properties of meningococcal Opa proteins were examined through their effects on proliferation of CD4+ T cells and secretion of pro-inflammatory cytokines in vitro.

**Results:** SDS-PAGE and MALDI-TOF mass spectroscopy were used to confirm purity and identity of purified Opa proteins. All 14 Opa proteins elicited bactericidal antibodies against at least one meningococcal isolate with SBA titres higher when the Opa proteins were mixed with Freund's adjuvant than with aluminium hydroxide. The percentage of isolates in each clonal complex killed in the SBA was 71-100% dependent on the clonal complex. Serum samples that did not kill target meningococci in the SBA were shown to still contain anti-Opa antibodies by whole cell ELISA. Immuno-electron microscopy demonstrated the binding of antibodies to the surface of meningococcal isolates and cellular responses to the recombinant proteins are presented.

**Conclusions:** Immunization with a panel of recombinant Opa proteins induces bactericidal antibodies against the majority of meningococci from a collection representing the hyperinvasive lineages. The immunomodulatory effects of Opa require further study.

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**C-8: FUNCTIONAL ACTIVITIES OF ANTIBODIES INDUCED BY PEPTIDE MIMETICS OF *N. MENINGITIDIS* SEROGROUP B CAPSULAR POLYSACCHARIDE**

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The capsular polysaccharide from *Neisseria meningitidis* serogroup B (CPS-B) shares some antigenic determinants with carbohydrates occurring as part of certain human tissues. However, it has been demonstrated the presence of a unique epitope, only found in the capsule layer of meningococcus. The possibility to direct the immune response toward this epitope is an alternative in the development of a safe and truly universal vaccine to fight the meningococcal B disease. A murine monoclonal antibody (mAb), with bactericidal and protective activity against *N. meningitidis* serogroup B and specifically recognizing a unique epitope in the meningococcal CPS-B [1], was used to select four peptide sequences able to compete with purified CPS-B for mAb binding. In the present study, functional activity of murine anti-peptide antibodies was studied by means of serum bactericidal assay and infant rat passive protection assay. The antibodies elicited against two of the peptides were able to kill *N. meningitidis* serogroup B cells and passively protect infant rats against challenge with live meningococci. Possible additive or synergistic effects of anti-peptide sera with anti-OMV sera were studied. Equal volumes of pooled sera from mice immunized with one of the peptides and pooled sera from mice immunized with OMV from serogroup B strain CU385 were mixed and tested. The serum bactericidal titers against CU385 were slightly higher when tested with mixed sera than with pooled sera alone. The peptides identified in this work could be considered as candidates for the formulation of vaccines against *N. meningitidis* serogroup B.

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**Symposium II.A: Current and New Neisserial Vaccines**

**KNA-3: MENINGOCOCCAL VACCINES; -STATUS AND ONGOING DEVELOPMENT STRATEGIES**

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For fighting and controlling the devastating meningococcal disease, it is only vaccines that can offer significant public health impact and relief of parents' fear. For the first time in history we are approaching a situation where meningococcal vaccines will be available for all epidemiological situations. Induction of circulating antibodies against the capsular polysaccharide has been a well proven strategy for important serogroups as A, C, Y, W-135. Recently, the principle of making effective protein-polysaccharide conjugate vaccines has been shown successful for these four serogroups. Thus, providing the option of having effective vaccines, that will be boostable and offers protection of longer duration (also in infants). However, for fighting serogroup B meningococcal disease the most promising options are to use sub-capsular antigens as the immunogenic target. A number of universal, cross-reactive antigens have been identified through “reverse vaccinology” and successfully tested as various formulations of recombinant protein vaccines. Currently one formulation is evaluated in a multi-centred Phase III trial. Conjugate vaccines
Bacterial meningitis caused by Neisseria meningitidis continues being a significant health problem in many areas in the world. This microorganism is divided into 13 serogroups based in structural differences in its capsular polysaccharide. The majority of the cases are caused by A, B, C, Y and W135 serogroups. There are several vaccines against A, C, Y and W135 serogroups which have been used as plain or conjugated polysaccharides, but against serogroup B the main strategy has been the use of Outer Membrane Vesicles as vaccine antigens. Finlay Institute has acquired a great experience in meningococcal vaccine field. Our leader product, VA-MENGOC-BC® has been extensively applied for more than 20 years and it is included in the Cuban National Immunization Program since 1991. This vaccine has controlled meningococcal disease in Cuba, with the decrease in morbidity (93%) and mortality (98%). In the last two years the institution has been involved in the production of polysaccharide A and C, to be supply for other countries, mainly for the Meningitis Belt Region in Africa. Recently Finlay Institute has started a collaboration project with the National Public Health Institute in Oslo, Norway, in order to obtain an Outer Membrane Vesicle vaccine, upon the base of the established technology use in Cuba to produce the serogroup B vaccine, but against A and W135 serogroups, taking into account that OMV vaccines induce immunological memory and bactericidal antibodies and they have shown very good immunogenicity and safety profiles with high stability. This preparation could be an alternative vaccine to be use for epidemic control.

C-1: MENINGOCOCCAL VACCINES AT FINLAY INSTITUTE: AN OVERVIEW

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Bacterial meningitis caused by Neisseria meningitidis continues being a significant health problem in many areas in the world. This microorganism is divided into 13 serogroups based in structural differences in its capsular polysaccharide. The majority of the cases are caused by A, B, C, Y and W135 serogroups. There are several vaccines against A, C, Y and W135 serogroups which have been used as plain or conjugated polysaccharides, but against serogroup B the main strategy has been the use of Outer Membrane Vesicles as vaccine antigens. Finlay Institute has acquired a great experience in meningococcal vaccine field. Our leader product, VA-MENGOC-BC® has been extensively applied for more than 20 years and it is included in the Cuban National Immunization Program since 1991. This vaccine has controlled meningococcal disease in Cuba, with the decrease in morbidity (93%) and mortality (98%). In the last two years the institution has been involved in the production of polysaccharide A and C, to be supply for other countries, mainly for the Meningitis Belt Region in Africa. Recently Finlay Institute has started a collaboration project with the National Public Health Institute in Oslo, Norway, in order to obtain an Outer Membrane Vesicle vaccine, upon the base of the established technology use in Cuba to produce the serogroup B vaccine, but against A and W135 serogroups, taking into account that OMV vaccines induce immunological memory and bactericidal antibodies and they have shown very good immunogenicity and safety profiles with high stability. This preparation could be an alternative vaccine to be use for epidemic control.

C-2: CONTROL OF NEW ZEALAND’S EPIDEMIC OF MENINGOCOCCAL DISEASE USING MENZB VACCINE

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Introduction: MeNZB vaccine was developed by Chiron (Novartis) specifically for the purposes of epidemic control. From 1991 New Zealand had experienced an increasing number of meningococcal disease cases caused by a strain, B:4:P1.7-2,4 not identified in New Zealand prior to 1990. The highest rate of disease (17.4/100,000) occurred in 2001. Methods: In 2001, a contract was signed with Chiron (Novartis) Vaccines to design a strain-specific vaccine for epidemic control. Following age-group trials demonstrating safety and immunogenicity MeNZB vaccine was delivered to individuals aged <20 years. Rollout of MeNZB began in July 2004 and continued through 2006. Results: Based on the immunization register close to 1.02 million individuals received three or more doses of MeNZB vaccine. Vaccine coverage for children aged <5 years was 84.8% and for those 5-17 years was 86.8%. In the pre-vaccination years 2002-2003, 407 (17.3 per 100,000) epidemic strain cases occurred in those aged <20 years compared with only 129 cases (5.4 per 100,000) in the post-vaccination years 2005-2006. Rates of disease fell markedly in those aged <5 years. Pre-vaccination case numbers in children <5 years in 2003 were 76 (28.1/100,000) whereas only 30 cases (10.9/100,000) <5 years occurred in 2006 and 23 cases (8.4/100,000) in 2007. Conclusions: Across all age-groups, epidemic strain disease has decreased. In 2008 case numbers totaled 100 cases, but only 44 were the epidemic strain type. Although the epidemic strain continues to dominate disease rates particularly in infants, it is unlikely that the epidemic strain would have undergone such rapid decline without the assistance of MeNZB vaccine.
C-3: LESSONS LEARNED FROM THE DEVELOPMENT OF A MENINGOCOCCAL DISEASE VACCINE BASED ON NEISSERIA LACTAMICA

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We have developed a candidate meningococcal disease vaccine based on outer membrane vesicles (OMV) from Neisseria lactamica. *N. lactamica* colonises the human nasopharynx, particularly in young children and its presence has been implicated in naturally-acquired meningococcal immunity. In pre-clinical studies, immunisation with *N. lactamica* OMVs protected mice from lethal challenge with *N. meningitidis* but without a detectable bactericidal antibody response. We have completed a phase I safety and immunogenicity study in adult volunteers. The vaccine showed a good safety profile and was immunogenic, eliciting large rises in antibody against the vaccine OMV and also against OMVs isolated from a panel of meningococcal strains. Modest increases in SBA titre were observed but a greater number of subjects showed increases in OPA activity. However, the results indicate that this is unlikely to be a successful meningococcal disease vaccine alone but it may provide a safe and immunogenic basis for a combination vaccine. A number of valuable lessons have been learned during this process. The transition from laboratory to GMP vaccine was challenging and appropriate advice should be sought at all stages. This study illustrated the value of using standardised assays using a defined strain panel that has been used to assess other vaccines, allowing direct comparison of results. The study also showed that high levels of pre-existing immunity must be taken into account in studies in adults, particularly if an epidemiologically relevant strain panel is used.

C-4: A BROADLY PROTECTIVE OMV VACCINE AGAINST MENINGOCOCCAL DISEASE FOR THE AFRICAN MENINGITIS BELT

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Introduction: *Neisseria meningitidis* is responsible for recurring epidemics of meningitis on the African continent. These epidemics are mainly caused by serogroup A, PorA serosubtype P1.20,9 strains of the ST-5 clonal complex, and partly by serogroup W-135 of the ST-11 clonal complex. In addition, the incidence of serogroup X disease has increased in the latest years, and there is no vaccine against this bacterium. The majority of African serogroup W-135 disease isolates harbor the PorA serosubtype P1.5,2 and most serogroup X and Y strains express the P1.5 epitope. We aim to develop the first affordable vaccine protecting against all these serogroups of meningococci, by developing a vaccine based on outer membrane vesicles (OMVs).

Material and methods: Phenotypic and genotypic characterizations of epidemic meningococcal disease isolates from the meningitis belt were undertaken to enable up-to-date information on the major target strains and guide selection of vaccine production strains. Studies on convalescent sera from Ethiopian serogroup A patients were performed to elucidate critical components of protective antibody responses induced during natural disease. OMV vaccines from serogroup A and W-135 strains were developed using similar methods as for the serogroup B meningococcal vaccines, MenBvac and MeNZB. The quantity, functional activity and specificity of the antibody responses in mice generated by a mixed A+W-135 OMV were analyzed. Results: Epidemiological studies confirmed the relatively low antigenic variation in major outer membrane proteins of the disease causing strains, allowing the use of OMV vaccines. Studies of the Ethiopian sera showed that outer membrane antigens were associated with the generation of putatively protective serum bactericidal assay (SBA) titers following disease. The vaccine induced antibodies in mice with high SBA against strains from different African countries, with antibodies being mainly directed towards the PorA and NadA outer membrane proteins. Conclusions: Immunization with the A+W-135 OMV vaccine candidate could probably protect against meningococcal disease caused by dominating strains in Africa. Production of clinical lots and a phase I trial in Cuba is planned.
C-5: NONAMEN, A MULTIVALENT MENB OMV VACCINE, FROM DEVELOPMENT TO CLINICAL STUDIES

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Meningococcal disease in most Western countries is mainly caused by serogroup B for which, in contrast to the successful introduction of Men C conjugate vaccines, still no effective vaccine is available. Outer membrane vesicle (OMV) vaccines are the only experimental vaccines against MenB tested in large phase III clinical trials. The used monovalent wildtype-OMV vaccines gave only protection against isolates expressing the identical outer membrane proteins. For a broad coverage against a variety of MenB serosubtypes, a multivalent OMV vaccine has been developed by the NVI. NonaMen consists of OMV’s of three different trivalent Neisseria meningitidis strains: HP16215, HP10124 and HP1416. Potential coverage of NonaMen in Europe was estimated 80% (Trotter and Ramsay, 2007). The production process for NonaMen has been optimized, preclinical lots for immunogenicity and toxicity studies and clinical lots for phase 1 study have been prepared. NonaMen has been shown to be immunogenic in mice and rabbits. Furthermore, NonaMen was tested in adult volunteers in a phase 1 clinical study to assess safety of a single dose of two different concentrations. All treatments were well tolerated and no systemic effects were found. The next step will be a multi dose safety and immunogenicity trial in adults. The establishment of immunologic correlates of protection is important for predicting vaccine effectiveness. It allows new vaccines to be licensed on the basis of attainment of defined immunologic benchmarks, without the need for large-scale efficacy trials for each new product. For group B the proportions of vaccinees with ≥4 fold rises in SBA pre- to post-vaccination or SBA titers ≥4 have been correlated with clinical efficacy in trials of OMV vaccines in Cuba, Brazil and Norway. It is anticipated that MenB vaccines will be licensed on the basis of immunogenicity data alone (SBA) without direct evidence of efficacy.

C-6: CLASSIFICATION OF NEISSERIA MENINGITIDIS IN RELATION TO VACCINE DEVELOPMENT

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Vaccines to prevent meningococcal disease caused by capsular serogroup A, C, Y, and W135 have been available for several years. The serogroup B capsule on the other hand is poorly immunogenic, making polysaccharide-based approaches not feasible to combat the main cause of meningococcal disease in industrialized countries. Therefore, the focus of vaccine development to protect against serogroup B disease has shifted to non-capsular approaches, including outer membrane vesicles, lipopolysaccharides (LPS), and various target proteins. Traditionally, in addition to the capsular polysaccharides, meningococcal strains have been classified on the basis of variation in their major outer membranes proteins, PorA and PorB, and their LPS. In the course of the past 10 years, molecular methods, including multilocus sequence typing (MLST), and porA and fetA sequencing, have become the tools of choice for strains characterization. While these techniques are extremely powerful for epidemiological surveillance, the information they provide may not be the one required to estimate the coverage and predict the impact of the serogroup B vaccines currently under development. Because of the propensity of the meningococcus to change its genome through uptake of DNA from close relatives, there is little association between variation in genes used for epidemiological studies and those targeted by the new vaccines. Therefore, analysis of sequence variation in the genes coding each of the potential vaccine antigens in representative strain collections, as well as measuring the level of expression of the target antigens, are necessary in order to determine the potential effect of these vaccines.

C-7: A GENOTYPIC COMPARISON OF INVASIVE AND NON-INVASIVE MENINGOCOCCI IN SCOTLAND 1996-2006. THE IMPACT OF THE MENC CONJUGATE VACCINE IN SCOTLAND

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Introduction: This presentation uses the extensive library of strains collected at the SMPRL to compare invasive and non-invasive meningococcal pre, during and post MenC conjugate vaccine implementation in the UK. Material and Methods:
MLST and paraA sequencing was performed on both invasive (IV) non-invasive (NIV) (i.e. carried) meningococci received by the SMPRL between 1996 and 2006. In addition genogrouping PCR and the capsule null locus (cnl) PCR was performed on all isolates. **Results:** >90% of IV isolates were serogroup B (58%) or C (34%); Only 37.5% of NIV isolates were serogroup B or C (36%, 1.5% respectively); 48% of NIV meningococci were non-groupable compared with 1.4% of IV isolates; The major MLST complexes ST-11/ET-37, ST-32/ET-5, ST-41-44/Lineage 3 and ST-269 were identified more often from cases of IV than in NIV; The major MLST complexes ST-213, ST-53, ST-35 and ST-23/Cluster A3 were identified more often in the NIV isolates than from cases of IV; NIV meningococci = 25, 40 and 10 VR1, 2 and 3 variants respectively; IV meningococci = 31, 41 and 10 VR1, 2 and 3 variants respectively. **Conclusions:** Nearly 50% of NIV meningococci were non-groupable; Serogroup C isolates were seldom found in the carriage population; MLST identified several of the so-called hypervirulent lineages amongst the NIV meningococci; including ST-269, ST-41-44 complex/lineage 3 and ST-213; The ST-53 complex was a major lineage amongst NIV isolates that was not found to cause IV.

**Symposium IIB: Current and New Neisserial Vaccines**

**KNA-4: A NEW GROUP A MENINGOCOCCAL CONJUGATE VACCINE (MENAFRIVAC™): PRIMARY PREVENTION TO END MENINGITIS OUTBREAKS IN AFRICA**

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**Introduction:** Epidemic Group A meningococcal meningitis continues to be a major public health problem in Sub-Saharan Africa. Waves of epidemic Group A meningococcal meningitis occur periodically in Africa and are superimposed on high endemic rates of disease. Reactive vaccination campaigns using meningococcal PS vaccines have not eliminated these epidemics. **Material and Methods:** The Meningitis Vaccine Project (MVP), a partnership between WHO and PATH, was established in 2001 with Gates Foundation support with the goal of eliminating epidemic meningitis through the development and widespread introduction of an affordable Group A meningococcal (MenA) conjugate vaccine. Using an innovative partnership model (Serum Institute of India; CBER/FDA, Bethesda and SynCoBioPartners, Amsterdam) MVP has developed a Men A conjugate vaccine (MenAfriVac™) with a target price of less than $US 0.50 per dose. **Results:** Preclinical development finished in 2004 and the vaccine has been successfully tested in Phase I and Phase II and II/III clinical trials in India and Africa (1-29 years). The vaccine has been shown to be safe, highly immunogenic and able to prime immunological memory when compared to polysaccharide vaccine. Widespread use of the vaccine is expected to generate broad herd immunity. **Conclusion:** MVP has successfully developed and managed a “push” strategy for the development of an affordable new vaccine product that was of little interest to major vaccine manufacturers. Introduction of the vaccine at public health scale is planned for 2009/2010 and after widespread introduction MenAfriVac™ is expected to eliminate Group A meningococcal meningitis epidemics from Sub Saharan Africa.

**C-1: CONJUGATED VACCINES AGAINST SEROGROUP A, C, W-135 AND Y DISEASE**

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Licensure of meningococcal C conjugate vaccines represented an enormous progress in the possibility of controlling meningococcal C disease. These vaccines have shown themselves highly effective, with a dramatic and immediate reduction in the incidence of meningococcal disease caused by serogroup C in countries that have introduced them in their mass immunization programs. Combination conjugate vaccines, containing more than one meningococcal polysaccharide, have been developed to broaden protection against the disease. A comprehensive overview of data on the currently available A-C-W135-Y meningococcal vaccine and the results of late stage development of novel tetravalent candidate vaccines will be reviewed. A tetravalent meningococcal vaccine that contains 4 ug each of capsular polysaccharide from serogroups A, C, Y, and W-135 conjugated to 48 ug of diphtheria toxoid (Menactra®- sanofi-pasteur) licensed on the basis of non-inferiority to the existing tetravalent polysaccharide vaccine is available only in USA and Canada for use in persons 2-55 years of age. However, in infants (the age group with the highest incidence rates of disease) the vaccine proved to be not immunogenic and is therefore not licensed for
use in children younger than 2 years. Currently, there is no broadly protective A, C, W-135, and Y conjugate vaccine licensed outside USA and Canada. A novel tetravalent meningococcal vaccine (ACWY) conjugated to CRM-197 (developed by Novartis) is being evaluated in Phase III trials and has demonstrated to be immunogenic and well tolerated in all age groups, including infants, representing a real possibility of a broader protection against meningococcal disease.

**C-2: DE-N-ACETYL SIALIC ACID-CONTAINING VACCINES FOR THE PREVENTION OF DISEASE CAUSED BY NEISSERIA MENINGITIDIS GROUP B AND C**

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Introduction: *Neisseria meningitidis* express polysialic acid (PSA) antigens that contain de-N-acetyl sialic acid (i.e. neuraminic acid-containing PSA or NeuPSA). In this study we evaluated the immunogenicity of vaccines based on NeuPSA derivatives and the protective functional activity of anti-NeuPSA antibodies against genetically diverse strains from all capsular groups. **Methods:** Antibodies were produced in CD1 mice by immunization with vaccines containing NeuPSA derivatives. Antibody specificity was determined by ELISA, binding to bacteria by flow cytometry, and functional activity by activation of complement, serum bactericidal activity (SBA), and human and infant rat models of meningococcal bacteremia. **Results:** Based on the reactivity of anti-NeuPSA mAbs, all strains of *N. meningitidis* appear to be able to express NeuPSA, or possibly cross-reacting antigens, regardless of capsular group. However, NeuPSA is most highly expressed in group B, C, W135, and Y strains that are cultured in chemically defined media (CDM), human serum or CDM supplemented with human serum. Interestingly, in strains from capsular groups other than B and C, expression of NeuPSA antigens is suppressed when grown in media containing non-human animal-derived supplements. NeuPSA-based vaccines enriched for non-reducing end Neu residues elicit IgG antibodies of all subclasses that bind to bacteria. Anti-NeuPSA antibodies mediate SBA, and are protective in models of meningococcal bacteremia against group B and C strains but not against strains from other capsular groups despite showing strong binding and complement activation. **Conclusion:** NeuPSA-based vaccines have the potential to protect against all group B and C strains.

**C-3: NEW GENERATION OF OUTER-MEMBRANE VESICLES BASED MENINGOCOCCAL VACCINES**

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With the development of conjugate vaccines, *Neisseria meningitidis* serogroup B (MenB) will remain the major cause of meningitis. In order to develop a vaccine able to confer a broad cross-protection against invasive MenB strains, we have developed a new generation of outer-membranes vesicles (OMVs). In order to avoid the dominant immune response mediated by PorA, we have genetically modified strain H44/76 to down-regulate the expression of this major variable outer membrane protein (OMP) and to up-regulate the expression of selected minor OMPs. The bactericidal response measured in sera form mice immunized with OMVs over-expressing only one minor OMP showed no or only limited increase in bactERICidal antibodies even if the induction of anti-specific OMP was demonstrated by ELISA. However, immunized mice with OMVs over-expressing different minor OMPs have induced the production of antibodies able to induce complement mediated killing of different meningococci strains. A safe LOS-based OMV vaccine was also developed using genetically modified strain producing a detoxified lipid A (deletion of msbB gene). After Extraction using low percentage of DOC, mice immunized with these OMVs produced bactERICidal antibodies direct against the LOS. In addition, we have also demonstrated that LOS lacking the terminal galactose (*lgtB* mutation – Trl3 LOS), but not galE LOS, induced a bactERICidal antibody response in mice similar to that seen for LOS containing the full Lacto-N-neoTetraose (L3,7 LOS). A bivalent LOS OMV vaccine based on L3,7 LOS and L2 LOS demonstrated the potential to afford a broad cross-protection against meningococcal disease.
C-4: APPLIED REVERSE VACCINOLOGY: AN INVESTIGATIONAL MENINGOCOCCAL SEROGROUP B VACCINE FOR INFANTS

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Introduction: Although strains of serogroup B N. meningitidis are the predominant disease causing strains in many regions of the world today, there is no truly global vaccine available to prevent this particular meningococcal infection. In a technique known as “reverse vaccinology,” potential vaccine candidates were predicted by analysing the entire genome sequence of an invasive MenB strain. Through genetic engineering and from the investigation of 350 potential antigens, surface proteins were identified that best induced an antibody response. Building on this genomics approach, it was possible to develop an investigational vaccine (rMenB) that offers protection against multiple strains of MenB, as measured by serum bactericidal antibody (SBA) assay. Phase II trials have demonstrated satisfactory safety, tolerability and immunogenicity. In addition, rMenB is the first recombinant MenB protein vaccine to induce an immune response in infants. Methods: Safety and immunogenicity of the rMenB vaccine was assessed in a 2, 4 and 6 month schedule. The immunogenicity was measured by SBA assay using human complement (hSBA). Results: The trial demonstrated satisfactory safety, tolerability and immunogenicity of the vaccine. Local and systemic reactions of the vaccine candidate were similar in frequency and intensity to routine infant immunisations with the exception of fever, which was reported more frequently in the rMenB vaccine recipients. A moderate, short-lasting temperature rise, not exceeding 39.0°C following the first dose, was reported more frequently in the rMenB vaccine arm than in the control. Immunogenicity analysis shows 89% (44/76-SL, ST32), 96% (5/99, ST8) and 85% (NZ98/254, ST41/44) hSBA =1:4 post third dose against three serogroup B strains representing the major vaccine antigens. The majority of disease causing strains worldwide express at least one of the MenB vaccine antigens. The immune response further increased with the fourth dose of the rMenB vaccine at 12 months (hSBA =1:4 post booster: 100% (44/76-SL), 98% (5/99) and 93% (NZ98/254)). Conclusions: rMenB vaccine is well tolerated and immunogenic against a panel of serogroup B strains in young infants when administered in a three dose schedule two months apart. This vaccine is entering phase 3 clinical trials.

C-5: NEISSERIA GONORRHOEAE PEPTIDE VACCINE CANDIDATE THAT MIMICS LIPOOLIGOSACCHARIDE DERIVED CARBOHYDRATE ELICITS COMPLEMENT-DEPENDENT KILLING ANTIBODIES THAT OVERCOME SERUM-RESISTANCE OF GONOCOCCI: PROPOSED TESTING IN A MOUSE MODEL OF INFECTION

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Introduction: Protective immunity to gonococcal infection does not occur after infection because broadly cross-reactive complement (C)-dependent antibody (Ab) killing does not prevail and persist. Mechanisms that prevent gonococcal killing include: (a) blocking Ab that interferes with binding of killing Ab; (b) sialylation of gonococci that facilitates binding of alternative C pathway regulator, factor H, and (c) binding of classical C pathway regulator, C4 binding protein (C4BP). Factor H and C4BP binding (and resulting function) is specific to humans. Materials and Methods: We characterized a carbohydrate (CHO) component of gonococcal lipooligosaccharide (LOS) present in 95% of clinical isolates that elicits killing antibody following infection. Because CHO antigens are poor immunogens, we identified a peptide mimic as a surrogate of the CHO epitope by screening a peptide display library with a monoclonal antibody (Mab) against the epitope. We configured a multiple antigenic peptide, immunized mice and tested antibodies for killing. Separately, we administered human C4BP to mice in an infection model and measured survival of gonococci. Results: Mouse immune antibodies killed gonococci, including sialylated organisms, thereby overcoming intrinsic serum resistance of N. gonorrhoeae caused by C regulators, factor H and C4BP. Mab specific for the epitope also overcame blocking antibody and killed gonococci. C4BP administration to mice prolonged infection and yielded greater colony counts throughout the period of infection. Conclusion: A peptide mimicking a LOS derived CHO antigen will serve as a potential gonococcal vaccine candidate in a mouse model that simulates the interaction of the human C system with N. gonorrhoeae.
In order to attain a state of establishment/persistence, meningococci use multiple surface antigens for adhesion and immune evasion in the mucosal environment. To understand how meningococci colonise the human respiratory sites, our studies have focussed on the structure-function relationships of surface adhesins and mechanisms of their interactions with host molecules. As with many mucosal bacteria, meningococcal adhesion is a complex process, involving several adhesins that may bind to distinct receptors of the host. Adhesins may act in concert and, through interacting with a variety of surface receptors, can lead to high avidity interactions, which often result in cellular invasion and tissue penetration. *N. meningitidis* pili, regarded as the most important adhesins in capsulate bacteria, may be aided by outer membrane opacity proteins for cellular invasion; and in the absence of pili and capsule, the opacity proteins act independently as effective invasins. In addition, a number of newly described minor adhesins may also play specific roles under *in vivo* conditions. In recent years, besides assessing the direct colonisation-related roles of the surface adhesins, we have analysed their influence on bacterial ability to overcome host innate and adaptive immunity. The aim of this presentation is to provide a brief update highlighting the dynamics of the adhesins at the mucosal epithelium, which may involve a number of host cell types. In addition, data will be presented from studies interrogating the potential influence of the adhesins in the context of host susceptibility to meningococcal infection, as well as their influence in the context of immune evasion (e.g. involving T cells, complement).

*Neisseria meningitidis* is a commensal of the human nasopharynx that in some circumstances can invade the bloodstream, cross the blood brain barrier and invade the meninges. The bacteria enter the central nervous system following a direct interaction with the luminal side of the cerebral endothelium, which constitutes the blood–brain barrier. To breach the barriers protecting the brain, *N. meningitidis* must cross a monolayer of tight junction-expressing endothelial or epithelial cells. The limited number of pathogens capable of crossing these tight barriers and invading the meninges suggests that they display very specific attributes. For *N. meningitidis*, type IV pili have been identified as being essential for meningeal invasion by inducing the formation of microvilli like structures on the apical surface of the endothelial cells. Meningococcal adhesion triggers a signalling leading to the formation of cortical plaques. These cortical plaques are enriched in ERM proteins (Ezrin, Moesin, Radixin), cortactin, actin, ErbB2 and adhesion molecules like ICAM-1/2 and VCAM-1. This signal leads to the formation of the microvilli-like strutures. Early work using epithelial cells suggested that *Nm* was capable of crossing a monolayer of tight junction forming cells using the transcellular route. However the lack of appropriate *in vitro* models of human blood brain barrier (BBB) did not allow determining the consequence of *Nm* interaction on brain endothelial cells. Using a well-differentiated human brain endothelial cell line hCMEC/D3, which closely mimics the BBB, we studied how meningococcal adhesion modifies the integrity of the junctional complexes in brain endothelial cells. We demonstrate that a bacterial pathogen like *Nm*, recruits the polarity complex Par3/Par6/PKCζ in a Cdc42 dependent manner, and hijacks the recruitment of adherens junction proteins. Furthermore we show that this specific targeting requires the previous organization of cortical actin following the recruitment of p120-catenin. Altogether these data suggest that *Nm* adhesion onto endothelial cells mimics spot-like adherens junction known to be recruited at nascent cell-cell contacts and is a consequence of a signalling similar to that involved in cell polarization and apical junctional complex assembly. This redistribution of the adherens junction molecules is likely to allow *Nm* to cross the BBB using the paracellular route.
**C-2: NATURALLY OCCURRING LIPID A MUTANTS IN NEISSERIA MENINGITIDIS: EFFECT ON PATHOGENESIS AND CLINICAL COURSE OF MENINGOCOCCAL DISEASE**

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**Introduction:** Recognition of lipopolysaccharide (LPS) by the TLR4/MD-2 receptor triggers the innate immune system to produce proinflammatory cytokines. *Neisseria meningitidis* has been reported to produce LPS with a hexa-acyl lipid A region, which is a highly active TLR4 agonist. Indeed, meningococcal sepsis is generally seen as the prototypical endotoxin-mediated disease. We have previously shown that insertional inactivation of the lpxL1 or lpxL2 genes required for addition of secondary acyl chains leads to reduced endotoxic activity of meningococcal LPS and whole bacteria. The possibility that such mutations might also occur naturally was suggested to us by a report showing that the group Y strain HF13 was defective in signaling through the MyD88-independent TLR4 pathway when bacteria were tested with mouse macrophages. **Methods:** 171 isolates from patients with meningococcal disease, representing 23 clonal complexes (cc), were obtained from the Netherlands Reference Laboratory for Bacterial Meningitis (AMC/RIVM). In addition the isolates of 40 patients of which we had an isolate of the blood or CSF and an isolate from the throat were included. Finally, isolates of 254 adults with meningococcal meningitis (Dutch Meningitis Cohort Study) of which clinical data were available were also included in the study. Induction by whole bacteria of cytokines IP-10 in mouse J774 cells and IL-6 in human MM6 cells was determined by ELISA. **Results:** We analyzed the lipid A structure of strain HF13 by mass spectrometry and found it to be penta-acylated, suggesting a mutation in the lpxL1 or lpxL2 genes. In agreement with this, we found a frameshift mutation which had inactivated its lpxL1 gene. We next screened panels of clinical isolates for cytokine induction in mouse or human macrophage-like cells. Strains which gave clearly reduced cytokine induction were readily identified, and sequencing of their lpxL1 genes revealed mutations in all of them. Overall, these natural lpxL1 mutants were surprisingly common, occurring in ca. 13% of meningococcal disease isolates of all major serogroups. Several different mutations were found, including frameshifts in homopolymeric tracts, small deletions, insertion of IS1016 and missense mutations in highly conserved residues. Wildtype vs mutant lpxL1 alleles could also be found in isolates from different anatomical locations within a single patient. Next, we investigated the clinical correlate of these mutations in a prospective nationwide observational cohort study of 254 adults with meningococcal meningitis. Seventeen patients (7%) with meningococcal meningitis were infected with an lpxL1 mutant strain. These patients tended to be younger (median 21 yrs [IQR 19-30] vs 31 [19-51]; P=0.052) and tended to present with higher body temperature (38.9°C [38.3-39.8] vs 38.4°C [IQR 37.5-39.1]; P=0.052), but were significantly less likely to present with rash (5/16 [31%] vs 157/236 [67%]; P=0.006) and had higher corresponding thrombocyte counts (225 x10⁹/L [163-279] vs 162 x10⁹/L [123-212]; P=0.009). **Conclusions:** Meningococcal lpxL1 mutants occur naturally with an unexpectedly high frequency, suggesting an important role in virulence for the resulting low-activity LPS. Patients infected by lpxL1 mutant meningococci present less frequently with rash and with higher thrombocyte counts, consistent with reduced cytokine induction and less activation of tissue-factor mediated coagulopathy.

**C-3: NEISSERIA-COMPLEMENT INTERACTIONS: IMPLICATIONS FOR PATHOGENESIS AND VACCINE DEVELOPMENT**

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Complement forms a key arm of innate immune defenses against the pathogenic Neisseriae. Neisseriae have evolved sophisticated mechanisms to evade human complement. The lacto-N-neotetraose lipoaoligosaccharide (LOS) of both species can bear a terminal sialic residue, yet only gonococcal LOS sialylation confers high-level serum resistance. Gonococcal LOS sialylation enhances binding of the alternative pathway (AP) inhibitor, factor H (fH); concomitant expression of gonococcal PorB is required for increased fH binding. The PorB of several strains of *N. gonorrhoeae*, in particular strains that cause disseminated disease, can bind to fH (PorB.1A) and/or the classical complement pathway inhibitor C4BP (PorB.1A and select PorB.1B) independent of LOS sialylation. Meningococci, however, bind to fH directly through the lipoprotein fH-binding protein (fHbp), currently a leading protein vaccine candidate. Binding of fH and C4BP to Neisseriae and their ability to resist complement-dependent killing is, in most instances, human-specific and could in part explain host-specificity of natural Neisserial infections and higher bactericidal titers seen when nonhuman complement sources are used to evaluate vaccine-elicited Ab. Most meningococci isolated from the bloodstream or cerebrospinal fluid possess a polysaccharide capsule. Capsular polysaccharide
Gonorrhea is a growing Public Health problem worldwide because of emergence of antibiotic resistance and other associated sexually transmitted diseases. Following infection with N. gonorrhoeae, bacteria may ascend into the Fallopian tubes (FT) and induce salpingitis, a major cause of infertility. Infection of FT explants with gonococci in vitro has shown cell death characteristic of apoptosis within epithelial cells. Other studies have indicated that the cytokine tumour necrosis factor (TNF)-α plays a role in cell death during salpingitis. Apoptosis of primary epithelial cells cultured from human FT was observed in vitro following infection with low numbers of gonococci. By contrast, increasing numbers of bacteria inhibited apoptosis. Moreover, the presence of adherent and internalized gonococci was observed in viable epithelial cells and not with cells that exhibited apoptosis.

Current models assume a role for Pili in attachment of gonococci and the ensuing internalization and local inflammatory responses. However, observations of our group and others indicate that Pili-minus gonococci have the capability to infect the reproductive tract of women. This prompted us to examine the effect of Pili upon time course of infection of FT explants and the related cytokine/chemokine responses. Mucosal explants were co-cultured with suspensions of Pili- and Pili+ N. gonorrhoeae strains that in addition did not express the Opa proteins. Attachment and internalization of gonococci and levels of IL-1β, IL-6, TNF-α, IL-10, TGF-β, IL-8, GM-CSF, MCP-1, MIP-1β, and RANTES in culture media were examined up to 24 h after infection. Attachment was higher for Pili+ gonococci but no statistically significant differences were found in the number of gonococci inside the epithelium between both strains. Both strains induced the release of the inflammatory cytokines IL-1β and TNF-α, as well as of chemokines GM-CSF, MCP-1, and MIP-1β, but those effects were smaller for the Pili+ compared with the Pili- strain. Results demonstrate that neither Pili nor Opa proteins are essential for invasion of the human oviduct mucosa.

Results suggest new intervention strategies for prevention and treatment of the disease.

(CPS) regulates the AP. Purified groups A, C, W-135 and Y (but not B) CPSs inhibit AP C3 convertase formation. CPS expression on intact bacteria (including group B) may block C3 binding to its membrane targets. Interestingly, groups W-135 and Y CPSs may themselves bind to human C3. Differences in complement evasion strategies employed by the two Neisserial species may contribute to differences in their clinical syndromes.

**C-4: IMMUNE EVASION BY NEISSERIA MENINGITIDIS IN THE RESPIRATORY TRACT**

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Commensal colonisers of the upper respiratory tract (URT) mucosa have evolved mechanisms to circumvent clearance by the host immune system. Carriage studies have revealed that N. meningitidis (Nm) can colonise the host for months at a time without causing immunopathology. We have shown that Nm primes an adaptive immune response rapidly upon the onset of carriage, generating both T cell and B cell memory. This appears to be offset by mucosal Treg activity which limits this immunity promoting continued carriage. In contrast, N. lactamica appears to evade the adaptive immune system in the URT mucosa, failing to prime the development of immunological memory during the peak period of nasopharyngeal carriage in early childhood. NI induces the production of T cell-independent polyclonal IgM which resembles ‘natural’ antibody. The production of ‘natural’ antibody may be one mechanism by which NI maintains immunological ignorance in the host, shielding it from the adaptive immune system, thus promoting continued carriage. In early encounters with the meningococcus, due to its polyclonal reactivity, this immunoglobulin may cross-react with colonizing Nm on the nasopharyngeal epithelium, offering some protection against both immune clearance from the mucosal surface and invasive disease. Interactions between these *Neisseria* species may therefore be fundamental in maintaining immunological ignorance in the host. This may represent a common mechanism by which mucosal bacteria interact with the host to maintain normal homeostasis and tolerance.

**C-5: PARACRINE RESPONSE OF FALLOPIAN TUBE CELLS INFECTED IN VITRO WITH N. GONORRHOEAE**

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Gonorrhea is a growing Public Health problem worldwide because of emergence of antibiotic resistance and other associated sexually transmitted diseases. Following infection with *N. gonorrhoeae*, bacteria may ascend into the Fallopian tubes (FT) and induce salpingitis, a major cause of infertility. Infection of FT explants with gonococci in vitro has shown cell death characteristic of apoptosis within epithelial cells. Other studies have indicated that the cytokine tumour necrosis factor (TNF)-α plays a role in cell death during salpingitis. \(N.\) \textit{gonorrhoeae} in the URT mucosa, failing to prime the development of immunological memory during the peak period of nasopharyngeal carriage in early childhood. NI induces the production of T cell-independent polyclonal IgM which resembles ‘natural’ antibody. The production of ‘natural’ antibody may be one mechanism by which NI maintains immunological ignorance in the host, shielding it from the adaptive immune system, thus promoting continued carriage. In early encounters with the meningococcus, due to its polyclonal reactivity, this immunoglobulin may cross-react with colonizing Nm on the nasopharyngeal epithelium, offering some protection against both immune clearance from the mucosal surface and invasive disease. Interactions between these *Neisseria* species may therefore be fundamental in maintaining immunological ignorance in the host. This may represent a common mechanism by which mucosal bacteria interact with the host to maintain normal homeostasis and tolerance.

\(N.\) \textit{gonorrhoeae} strains that in addition did not express the Opa proteins. Attachment and internalization of gonococci and levels of IL-1β, IL-6, TNF-α, IL-10, TGF-β, IL-8, GM-CSF, MCP-1, MIP-1β, and RANTES in culture media were examined up to 24 h after infection. Attachment was higher for Pili+ gonococci but no statistically significant differences were found in the number of gonococci inside the epithelium between both strains. Both strains induced the release of the inflammatory cytokines IL-1β and TNF-α, as well as of chemokines GM-CSF, MCP-1, and MIP-1β, but those effects were smaller for the Pili+ compared with the Pili- strain. Results demonstrate that neither Pili nor Opa proteins are essential for invasion of the human oviduct mucosa. Pili appear to have a modulatory role during infection of the human tubal mucosa leading to slowing down of the initial stages of invasion and the associated inflammatory responses. Understanding the mechanisms used by *N. gonorrhoeae* will inform the pathogenesis of salpingitis and could suggest new intervention strategies for prevention and treatment of the disease.
C-6: MUCOSAL IMMUNE RESPONSE INDUCED BY PROTEOLIPOSOME AND COCHLEATE DERIVED FROM SEROGROUP B N. MENINGITIDIES


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Mucosal vaccination offers attractive advantages to conventional systemic vaccination. Most pathogens enter or establish infection at mucosal surfaces. This represents an enormous challenge for vaccine development. Nevertheless, the availability of safe and effective adjuvants that function mucosally is the major limitation. Therefore, we investigated the impact of mucosal immunization with the Neisseria meningitidis B proteoliposome (AFPL1, Adjuvant Finlay Proteoliposome 1) and its-derived cochleate (Co, AFCo1). They contain multiple PAMPs as immunopotentiators and have delivery system ability as well as Th1 polarization activity. Groups of female mice were immunized by nasal, oral, intravaginal, or intramuscular routes with three doses with AFPL1/AFCo1 alone or containing ovalbumin or glycoprotein (g) D2 from Herpes Simplex Virus type 2 (HSV-2). High levels of specific IgG antibodies were detected in sera of mice vaccinated with either route. However, specific IgA antibodies were produced in saliva and vaginal wash only following mucosal delivering. The polarization to a Th1 pattern was confirmed by testing the induction of IgG2a/IgG2c antibody, positive delayed-type hypersensitivity reactions, and γIFN production. Additionally, AFCo1gD2 showed practically no vaginal HSV-2 replication and 100% protection against lethal vaginal HSV-2 challenge. In conclusion, the results support the use of AFCo1 as potent Th1 adjuvant for mucosal vaccines, particularly for nasal route.

C-7: IMMUNE RESPONSES FOLLOWING EXPERIMENTAL COLONIZATION OF ADULT VOLUNTEERS WITH NEISSERIA LACTAMICA

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It is generally agreed that natural immunity to meningococcal disease develops following asymptomatic carriage of Neisseria meningitidis and related commensal Neisseria species. To study the development of immunity following carriage we have challenged adult volunteers with N. lactamica. 210 healthy adults were screened for Neisseria carriage and 138 non-colonised individuals were identified for the study. 41 were given a live intranasal challenge with 104 N. lactamica, using a GMP seed stock made from the HPA OMV vaccine strain. 20 subjects received a PBS control dose. Colonisation with the challenge strain and other Neisseria spp. was detected from culture of posterior pharyngeal swabs and gargle fluid. Specific mucosal and systemic antibody responses to N. lactamica were determined by ELISA and cross reactive functional antibody responses to a panel of meningococcal strains were determined using serum bactericidal antibody and opsonophagocytosis assays. 26 out of 41 (63.4%) subjects became colonised with N. lactamica. Three controls (15%) acquired N. meningitidis but none of those who received N. lactamica. Colonised individuals showed a rise in salivary IgA and total IgG to N. lactamica. A low grade bactericidal response was observed at 4 weeks and at 12 weeks against at least one of 6 meningococcal strains. Thus we have demonstrated that experimental colonisation with N. lactamica elicits mucosal and serum antibody to the inoculated strain and weak cross-reactive responses against the meningococcus which may be sufficient to restrict acquisition of meningococcal carriage over an extended period.
Introduction: New and improved vaccines against meningococcal disease are still needed. Although serum bactericidal activity (SBA) is the preferred laboratory correlate of protection (“The gold-standard”), low SBA titers (titres < 4 with human complement) do not necessarily predict susceptibility. Opsonophagocytosis (OP) may be an important effector mechanism for protection against meningococcal disease. However, the protocols for measuring OP against meningococci are not standardized and different methods may yield different results.

Material and Methods: Opsonophagocytic activity (OPA) was measured as respiratory burst against live serogroup A or serogroup B meningococci using human serum as complement source and human neutrophils as effector cells. Serial dilutions of sera from vaccinees were tested and the results were reported as the highest serum dilution giving opsonophagocytic activity of ≥ 50% of the neutrophils. Results from clinical trials of a serogroup B OMV vaccine (MeNZB) and a new meningococcal serogroup A conjugate vaccine (MenAfriVac) will be presented.

Results: Data from the serogroup B OMV vaccine study in adults indicate that opsonophagocytic activity correlates significantly both to the level of IgG antibodies against OMVs and to SBA. After 3 vaccine doses we observed 96–100% with a fourfold increase in OPA titer against the homologous strain, and 82-88% against a heterologous B strain, whereas the number of responders in hSBA was 50-53% (1). An effectiveness of 80% was observed in New Zealand against group B diseases in children aged under 5 years (2). Against serogroup A meningococci we observed a significant OPA response after vaccination of healthy adults, but the correlation to SBA with rabbit complement was very low. Conclusion: SBA probably underestimates protection against meningococcal disease. Using OPA against group B meningococci we observe more responders than are revealed by SBA, and also a higher degree of cross-reactivity against heterologous strains. OPA may add valuable information when performing vaccine trials.

References:
C-2: ALTERNATIVE COMPLEMENT SOURCES FOR SEROGROUP B SERUM BACTERICIDAL ANTIBODY ASSAYS

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The surrogate of protection against *Neisseria meningitidis* serogroup B (MenB) is the serum bactericidal antibody (SBA) assay which measures functional activity of antibody using exogenous complement. Early studies used human complement, but problems gaining sufficient quantity and quality resulted in a commercially available source becoming an attractive alternative. Consequently, rabbit complement has been used in serogroup A, C, Y and W135 SBA assays. However, it is not recommended for use in the MenB SBA assay due to elevated SBA titres caused by low avidity anti-MenB capsular antibody in test sera. Therefore, the possibility of utilising complement from other species has been investigated with varying degrees of success. These include complement from smaller species such as guinea pig and rat, although these generally perform similarly to rabbit complement. There is now growing evidence that complement from larger species such as Bovine and Porcine may produce SBA titres more similar to those gained with human complement. Another approach has been the addition of components such as human factor H in an attempt to humanise non-human complement. This was demonstrated to reduce serogroup C rabbit complement SBA titres, but requires further investigation. Addition of colominic acid to absorb anti-MenB capsular antibody from test sera was shown to reduce MenB rabbit complement SBA titres, but these were not always comparable to those gained with human complement. In summary, approaches to enable the use non-human complement in the MenB SBA assay are progressing and following evaluation/validation may be suitable for use in clinical studies.

C-3: MENINGOCOCCAL CARRIAGE AS AN ALTERNATIVE CORRELATE OF PROTECTION

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In industrialised countries, the highest incidence of meningococcal disease is observed in young children, while colonisation is most common in teenagers and young adults. The prevalence of meningococcal carriage is a poor predictor of disease risk because other factors, including host susceptibility and the invasive potential of the organism, are important. The natural history of meningococcal infection is dominated by transmission between carriers and disease is a relatively rare event. As the experience with meningococcal serogroup C conjugate (MCC) vaccines illustrates, vaccines that can influence transmission in addition to disease will have much greater population impact. In the UK, excellent control of serogroup C disease continues, largely because of sustained herd immunity. The ability of other meningococcal conjugate and candidate protein vaccines to reduce carriage, and indeed the effect of MCC vaccines in different epidemiological contexts, is not known. Given the potential magnitude of herd immunity that can be achieved, carriage studies should be considered as an important component of vaccine evaluation. Outcomes of interest include the prevalence of carriage before and after immunisation. This information is highly relevant in terms of optimising vaccine strategy and determining the likely cost-effectiveness of immunisation programmes. For example, if a vaccine has no effect on carriage, routine infant immunisation may be preferred, whereas for a vaccine that is able to prevent carriage, strategies that include a catch-up campaign and target the age-group which is driving transmission may be much more attractive.

C-4: EXPERIMENTAL DISEASE MODELS CURRENTLY USED FOR THE ASSESSMENT OF SEROGROUP B MENINGOCOCCAL VACCINES

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*Neisseria meningitidis* is a strict human pathogen, due to the specificity of several meningococcal surface proteins that interact with the host. Hence, there is no reliable animal model for meningococcal disease, and it has hampered the development of vaccines against meningococci. Immunization of experimental animals followed by serological assays is generally employed when determining the immunogenicity of vaccine candidates against serogroup B *N. meningitidis* (MenB). Analyses of complement C3 deposition on the surface of the bacterium and opsonophagocytic activity are often used in the assessment of the functional ability of the antibodies against MenB antigens. Nowadays, serum bactericidal activity has been accepted as the closest in vitro correlate of protection for protein-based serogroup B vaccines. However, most of the research and development groups
Additionally evaluate the protective capacity of the antibodies in animal models of meningococcal infection. In the absence of an optimal animal model, a diversity of experimental disease models has been explored. Among them are the widely used models of intraperitoneal infection in adult mice and infant rats. Attempts have also been done to develop intranasal infection models. The neonatal mouse model is also been used to evaluate the immunogenicity of vaccine candidates in an animal model that resembles the immaturity of the immune system of young children. In addition, transgenic mice expressing human proteins have been recently explored as mouse model for meningococcal infection. An overview on the experimental meningococcal disease models currently used for the assessment of serogroup B vaccines will be presented.

C-5: ALTERNATIVE APPROACH FOR ASSESSING EFFECTIVENESS OF MULTICOMPONENT RECOMBINANT PROTEIN MENINGOCOCCAL SEROGROUP B VACCINE IN MAN

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A multicomponent recombinant protein vaccine against meningococcus B that uses novel antigens discovered by whole genome sequencing, combined with OMV, is being evaluated in clinical trials. Serum bactericidal antibodies detected with human complement are widely accepted as a surrogate marker of resistance to meningococcal meningitis and this vaccine elicits a bactericidal response with broad crossreactivity in both laboratory animals and human subjects. Serum bactericidal results vary among the strains used in the assay as well as assay conditions and source of complement and consequently it is not straightforward to select a representative panel of strains to be used in the Serum Bactericidal Assay (SBA) for clinical trial samples. The functional activity against any given strain of MenB can reflect the combined effects of antibodies against many antigens. To assess the effectiveness of this multicomponent vaccine we decided to measure the bactericidal antibodies specifically induced by each major antigens fHBP, NadA and GNA 2132. We screened for isolates of MenB which matched the vaccine for individual components included in it. Next, we performed competitive bactericidal assays on serum from human vaccinees, by adding the soluble recombinant antigens contained in the vaccine into the bactericidal reaction, to determine whether killing of a particular strain by functional antibodies was inhibited by a specific antigen or combination of antigens. We identified a panel of MenB strains that when used as targets in the SBA each demonstrate that one of the major components is able to evoke a protective bactericidal response independently, and that recognition of any one of the components is sufficient to provide a bactericidal response. We found that most adult human subjects made bactericidal antibodies against each of the major components. The very great diversity of MenB makes it unlikely that a sufficient number of different MenB strains could be tested in the SBA on human subjects to assess the extent of crossreactivity among strains of the bactericidal response to this vaccine. The panel of strains that we have developed makes it possible to demonstrate the presence of a protective response against each major component. Therefore, a typing assay that detects the presence of the components of the vaccine on different MenB strains, provided that the results of such a method can be linked to killing of the strains in the SBA, could be used for demonstration of the effectiveness of the vaccine.

C-6: CORRELATION OF HIGH THROUGHPUT FLOW CYTOMETRY OPSONOPHAGOCYTOSIS AND ANTIBODY-MEDIATED MEMBRANE ATTACK COMPLEX ASSAYS WITH KILLING OPSONOPHAGOCYTOSIS AND BACTERICIDAL ANTIBODY ASSAYS

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Serum bactericidal activity has been established as a correlate of protection for polysaccharide-based meningococcal vaccines. However, for protein-based vaccines the correlates of protection are less clear. We have developed high throughput opsonophagocytosis and antibody-mediated complement deposition assays, although correlation with protection is currently undetermined. This study has aimed to correlate the responses from these qualified high throughput assays with more labour-intensive functional assays to ascertain their usefulness in clinical trial sera assessment. A high-throughput flow cytometry-based opsonophagocytosis assay (OPA) performed using BCECF-labelled fixed N. meningitidis, IgG-depleted human plasma and a DMF-differentiated HL60 granulocytic cell line was compared with an opsonic killing assay adapted from that reported by Pleased and Granoff (Clin Vacc Immunol 2008). Also, a high throughput antibody-mediated complement deposition assay using fixed meningococci, IgG-depleted human plasma, and fluorescent anti C3c and C5-C9 antibodies was correlated with a standard serum bactericidal assay. The assays were performed using a panel of human vaccinee sera and Pearson correlation coefficients determined. Good correlations (95% confidence) between deposition of C5b-9 and bactericidal titres were determined.
C-6: CORRELATION OF HIGH THROUGHPUT FLOW CYTOMETRY
OPSONOPHAGOCYTOSIS AND ANTIBODY-MEDIATED MEMBRANE
ATTACK COMPLEX ASSAYS WITH KILLING OPSONOPHAGOCYTOSIS
AND BACTERICIDAL ANTIBODY ASSAYS

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Serum bactericidal activity has been established as a correlate of protection for polysaccharide-based meningococcal vaccines. However, for protein-based vaccines the correlates of protection are less clear. We have developed high throughput opsonophagocytosis and antibody-mediated complement deposition assays, although correlation with protection is currently undetermined. This study has aimed to correlate the responses from these qualified high throughput assays with more labour-intensive functional assays to ascertain their usefulness in clinical trial sera assessment. A high-throughput flow cytometry-based opsonophagocytosis assay (OPA) performed using BCECF-labelled fixed N. meningitidis, IgG-depleted human plasma and a DMF-differentiated HL60 granulocytic cell line was compared with an opsonic killing assay adapted from that reported by Pleased and Granoff (Clin Vacc Immunol 2008). Also, a high throughput antibody-mediated complement deposition assay using fixed meningococci, IgG-depleted human plasma, and fluorescent anti C3c and C5-C9 antibodies was correlated with a standard serum bactericidal assay. The assays were performed using a panel of human vaccinee sera and Pearson correlation coefficients determined. Good correlations (95% confidence) between deposition of C5b-9 and bactericidal titres were determined and the high throughput opsonic assay correlated well (95% confidence) with the opsonic killing assay. We have also demonstrated strong correlation with opsonic responses and C3c deposition. These results show that these high-throughput assays performed on azide-fixed targets are useful in a large scale clinical serology testing.

Symposium V: Manufacture, Control and Regulation of Neisserial Vaccines

KNA-7: BATCH CONSISTENCY AND SAFETY TESTING OF MENINGOCOCCAL VACCINES

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NIBSC has been involved in the batch release of a number of meningococcal vaccines since the licensure of the first polysaccharide vaccine. More recently this has included polysaccharide conjugates and outer membrane vesicle (OMV) vaccines. The meningococcal serogroup C polysaccharide (MenC) conjugate vaccine was licensed in the U.K. in 1999. Principally physicochemical tests, such as molecular size determination, saccharide content and % free saccharide, are used to ensure that the products meet licence specifications and remain consistent with batches previously shown to be safe and immunogenic in clinical studies. The outer membrane vesicle vaccine MenZB introduced to control an outbreak of meningococcal disease in New Zealand was evaluated for safety and consistency at NIBSC. These vaccines are more complex than the MenC conjugate vaccine containing a complex mixture of outer membrane proteins. Many of these proteins are highly variable, a number showing phase variation, or their expression regulated by response to environmental stimul. Variability in the vaccine can result from changes in growth conditions during manufacture. One dimensional SDS polyacrylamide gel electrophoresis (SDS-PAGE) has typically been used to ensure consistency in the relative amounts of key antigens in an OMV by measuring the protein band intensities on a Coomassie stained gel. However, some OMV antigens are difficult to identify unequivocally using this approach. Two dimensional electrophoresis and fluorescent labelling have been used to identify the protein compliment of the MenZB vaccine and evaluate batch consistency. These techniques are impractical for routine use but highlight key proteins that could be monitored in future batch testing.
C-1: FACILITY RETROFITTING AND INDUSTRIAL GMP PRODUCTION OF POLYSACCHARIDE A AND C FROM *NEISSERIA MENINGITIDIES* FOR THE DISEASE IN AFRICA: A TECHNOLOGICAL CHALLENGE


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**Introduction:** In March 2007, Cuba received the demand from WHO to produce in a fast track project de polysaccharide A and C from *Neisseria meningitis* for the epidemic season in Africa, because in the pharmaceutical market the capacity for this product and the number of producers decreased. To solve this challenge the experience in Finlay Institute for industrial API production and in Bio-Manguinhos from Brazil for freeze-drying of products where joined to this goal. The timeframe for supplying was December 2007 and the total required doses of 12-15 millions. The product must be obtained under GMP production and a WHO prequalification process should occur before starting the supplying. **Results:** In this work we present two strategies: (1). The retrofitting and existing facility (30 m² of clean rooms) scale up and introduction of new disposables process technologies for fast process introduction and validation, that permit to pass the prequalification process and the approval for WHO for the supplying of this product in October 2007 and the successful production of 10-12 million of doses of this two polysaccharide and (2). In parallel to the first strategy, the design and building of a new complete facility (700 m² of clean rooms) for the production of 100 millions doses that will be in operation in 2009 to cover the demand market increase of this product. **Conclusions:** The supply of *N. meningitidis* A/C vaccine in a cooperation project between Finlay and Bio-Manguinhos was successful in the proposed time for the epidemic in Africa according with the WHO requirements.

C-2: TENDENCES FOR *N. MENINGITIDIS* CAPSULAR POLYSACCHARIDE PRODUCTION

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The polysaccharide (PS) capsule is one of the outermost structures of pathogenic *Neisseria meningitidis*; it has been a primary focus of attempts to develop vaccines. The capsular PS production has been enhanced, increasing yield by reducing stage in the whole process. In the upstream process, improved culture medium are used to support high-cell density rising bigger polysaccharide expression; on the other hand, the downstream or purification process have been modify from a “preparative” technology where capsular PS from *N. meningitidis* are typically prepared by a process comprising the steps of PS precipitation (e.g. using a cationic detergent), ethanol fractionation, cold phenol extraction (to remove protein) and ultracentrifugation (to remove LPS) to an “industrial” method by means of tangential flow filtration systems, depth filtration, chromatography and others, with a high capacity and resolution process, with a consequent step reduction and productivity increasing in order to get a competitive product.

**Keywords:** Polysaccharide; culture media, purification.
A-1: ENHANCEMENT OF ANTIBODY RESPONSE TO NEISSERIA MENINGITIDIS GROUP C POLYSACCHARIDE-P64K PROTEIN CONJUGATES BY THE MENINGOCOCCAL GROUP A PURIFIED CAPSULAR POLYSACCHARIDE AS ADJUVANT

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Introduction: The development of new adjuvants for human vaccines is an important and expanding field of research. Bacterial polysaccharides constitute a source of potential adjuvants that mediate its activity through activation of innate immune response receptors that mediate the danger signals activating the host immune defense system. Objectives: To evaluate the capacity of the purified capsular polysaccharide (CPS) from group A of N. meningitidis to enhance the immune response directed against the meningococcal serogroup C-CPS covalently conjugated to the carrier protein P64K (CPS-C/P64K). Materials and Methods: Groups of 6 Balb/c mice each were immunized subcutaneously with the conjugate and the conjugate mixed either with the CPS-A or the CPS-C. Levels of total IgG and IgG2a subclass were measured by Elisa using CPS-C as coating antigen. Functional activity of antibodies was detected by serum bactericidal assay. Results: The conjugate CPS-C/P64K mixed with CPS-A developed a greater IgG and IgG2a antibody response and higher anti-meningococcal bactericidal titers either than the conjugate or the conjugate mixed with CPS-C. Conclusions: The addition of the meningococcal polysaccharide A enhanced the antibody response to the N. meningitidis group C polysaccharide-P64K protein conjugates.

A-2: PREDICTED OUTER MEMBRANES PROTEINS OF NEISSERIA MENINGITIDIS AS POTENTIAL VACCINE CANDIDATES: FROM IN SILICO ANALYSES TO EXPERIMENTAL CORROBORATION

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Neisseria meningitidis serogroup B infections are a serious health threat to developed and developing countries. Sequence variation of surface-exposed proteins and cross-reactivity of the serogroup B capsular polysaccharide with human tissues have hampered efforts to develop a successful universal vaccine. Outer membrane vesicles-based vaccines available in the market, although effective in solving problems of epidemics, nor have represented a final solution to this problem. Currently, several novel outer membrane proteins have been identified through proteomics, immunoproteomics, and bioinformatic analysis of the genome and they have been further characterized as potential vaccine candidates. In this study, using an alternative in silico approach, we have selected a set of new potential outer membrane proteins that could also be exploited as vaccine components. A total of eight final candidates, that has not been characterized before, were cloned and expressed in E. coli. The resulting products were purified by Metal Chelating Chromatography and used in an immunization schedule in mice. Although all resulted immunogenic, bactericidal response was detected only in antisera elicited by six of them. The infant rat protection assay also gave positive results for four candidates, either against the homologous and/or the heterologous strains. Conservation analyses were also carried out sequencing selected genes in a panel of diverse meningococcal strains. Our study demonstrated that utilization of genome sequences by application of bioinformatics is still possible to expedite the vaccine discovery process in N. meningitidis by rapidly providing a set of uncharacterized candidates for further testing.
**A-3: NMB0181, AN IMMUNOGENIC PROTEIN THAT INDUCES BACTERICIDAL ANTIBODIES IN MICE AGAINST N. MENINGITIDIS SEROGROUP B**

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*Neisseria meningitidis* is a gram-negative encapsulated diplococcus that could cause epidemic meningitis with severe sequelae. Serogroup B meningococcus is the most important cause of endemic meningitis in industrialized countries, accounting for 30–40% of cases in North America and for 30–80% in Europe. Although several studies have been carried out to find a vaccine that protects against all circulating bacteria, there is not a universal vaccine in the market until now. New meningococcal antigens that could induce a cross-bactericidal response against the meningococcus, are emerging as a possible solution. In this study we described a novel antigen, a 19KDa protein that was selected by an *in silico* strategy and previously annotated as NMB0181 (putative outer membrane protein OmpH) in the published genome of serogroup B strain MC58. Computational analysis showed that this protein has some sequence similarities with bacterial haemolysins. The respective gene was amplified by PCR, cloned into the pM238 vector and expressed in *Escherichia coli* as inclusion bodies. After an efficient purification step by immobilized metal ion chromatography, the recombinant protein was used to immunize Balb/C mice. The antiserum obtained showed bactericidal activity against the homologous strain CU385 and it also recognized the native antigen in different strains by Western Blotting. Conservation analysis taking into account the information derived from the four sequenced neisserial genomes and the amplified gene from the CU385 cuban strain, showed a 98% of identity. According to our results, although a more diverse characterization is needed, NMB0181 protein emerges as a possible vaccine candidate to prevent meningococcal disease.

**A-4: SEQUENCE ANALYSIS OF THE NMB0088 GENE: VARIABILITY, PREDICTED PROTEIN TOPOLOGY AND ANTIBODY RESPONSE AFTER IMMUNIZATION WITH THE RECOMBINANT PROTEIN**

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After several proteomic studies, NMB0088 (or OmpP1) of *Neisseria meningitidis* appears to be abundant in outer membrane vesicle preparations. In the present work, we propose a structural model for this surface-exposed outer membrane protein and also evaluated if this antigen is subject to diversifying selection. Moreover, a recombinant variant of NMB0088 was evaluated in animal models included in a liposomal formulation. Our analysis suggested that NMB0088 is a beta-barrel protein with 7 exposed loops and 14 membrane spanning segments. Four variants of NMB0088 were identified among 45 strains and the variability was confined to three specific segments, designated VR1, VR2 and VR3. Secondary structure analysis, 3D structural database searches, and homology modeling using FadL of *Escherichia coli*, revealed that almost all the variable regions are located with in or near extracellular looping domains. Antiserum produced against the recombinant protein were capable of recognize a protein in Western blot analysis of some meningococcal strains from different serogroups and NMB0088 variants. Bactericidal and protective antibodies were detected against the homologous strain, but antisera were not able to kill one heterologous strain with a different variant of the protein. Accordingly, the antigen NMB0088 could be considered in future works as a vaccine candidate for a subunit recombinant vaccine against the meningococcus. However, the level of diversity of the NMB0088 exposed loops suggests that vaccine preparations based on this antigen should be carefully designed in order to provide broad coverage against *N. meningitidis*. 
**A-5: IMMUNOLOGICAL EVALUATION AND SEQUENCE CONSTANCY OF TWO NEW MENINGOCOCCAL VACCINE CANDIDATE PROTEINS**

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A growing number of high-throughput approaches have been employed to screen the meningococcal genome for potentially protective antigens. Recent developments in genomic technology, together with the availability of genome sequences provide the opportunity to examine such proteins, which might form the basis of a truly universal vaccine, if they were present on all strains irrespective of serogroup. In our laboratory, the novel meningococcal antigens NMA0939 and NMB0938 were identified by mining meningococcal genomic sequence databases. In this work, we evaluated these proteins as potential vaccine candidates to control meningococcal disease. The genes were present in 100% of the strains evaluated. It was found, after the sequence analysis, the overall identity of the deduced polypeptides ranged from 95 to 100%. When recombinant variants of the antigens were used as immunogens, animals developed cross-reactive IgG antibodies in their sera, as determined by ELISA and Western blotting using whole cells of homologous and heterologous strains. FACS analysis showed binding of mouse polyclonal sera to live N. meningitidis from the CU385 strain, suggesting that these proteins are exposed on the surface of the cells. Besides, the immunization induced a functional response characterized by bactericidal antibodies and protective activity against meningococcal bacteremia in the infant rat model. Taking into account these findings, NMA0939 and NMB0938 have several attributes that make them promising candidates to be included in a future vaccine against meningococcal disease.

**A-6: IMMUNOLOGICAL EVALUATION AND SEQUENCE CONSERVATION OF MENINGOCOCCAL ANTIGEN NMB0606**


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Computational prediction methodologies in conjunction with new proteomic tools are powerful methods for recognizing outer membrane proteins with potential as vaccine candidates. The application of both methodologies to Neisseria meningitidis allowed us to identify a novel 16 kDa molecule, previously annotated as NMB0606 (hypothetical protein) in the published genome of serogroup B strain MC58. To test the immunogenic properties of this protein, the corresponding gene was first cloned into the pM238 vector and expressed in Escherichia coli GC366 strain. An immunization schedule was carried on in Balb/C mice. Whole cell ELISA and surface labelling assays corroborated the outer membrane localization prediction for the protein NMB0606. In addition, antisera generated against the recombinant antigen showed to be bactericidal and protective against the meningococcus. The functionality of the elicited antibodies was in agreement with positive results obtained after C3 complement deposition experiments. Furthermore, phylogenetic analyses using a panel of 47 meningococcal strains showed this antigen is highly conserved, with only one polymorphic site and two alleles detected in the whole population under study. Our results suggest that this protein could be an attractive vaccine candidate in the prevention of meningococcal disease.

**A-7: IMPROVED IMMUNOGENICITY OF A GROUP B OUTER MEMBRANE VESICLE-BASED VACCINE AFTER THE INCORPORATION OF A RECOMBINANT ANTIGENS**


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In recent years, there has been a growing interest in developing meningococcal vaccines. The lack of a universal vaccine has led to the exploration of new approaches to improve the immunogenicity of existing vaccines. In this work, we evaluated the incorporation of a recombinant antigen into a group B outer membrane vesicle-based vaccine. The results showed an improved immunogenicity, as evidenced by the elicitation of higher levels of antibodies in the sera of vaccinated animals. This approach could potentially lead to the development of a more effective vaccine against meningococcal disease.
In order to increase the cross-immunogenicity of OMV vaccines several strategies have been explored, including the enrichment of OMV with minor conserved meningococcal antigens. We have previously reported the potential of a lipoprotein NMB0928, as a candidate for a cross-protective vaccine. However, NMB0928 is poorly represented in the OMV preparations as shown by immunochemical and proteomic assays. In this work we are introducing a method for the incorporation of the recombinant protein NMB0928 into serogroup B OMV. The new formulation was used to immunize mice using aluminum hydroxide as adjuvant and the sera were evaluated by immunoassays. After immunization of mice with the OMV vaccine overloaded with recombinant NMB0928, a broader cross-reactivity with diverse meningococcal strains was found for the antibodies generated against the complex, compared to the response detected for the antisera elicited against the OMV alone. Antiserum from mice immunized with the modified OMV vaccine also elicited greater deposition of human C3 complement on the surface of live N. meningitidis bacteria and greater protective activity against meningococcal bacteraemia in infant rats. This work demonstrates the feasibility of this strategy in eliciting improved antibody levels and a protective response against homologous and heterologous neisserial strains. Our method is also a solution to increase the amount of lipoproteins on the surface of OMV, since these antigens appear to be affected after the extraction steps with detergents.

A-8: SEQUENCE VARIABILITY AND PHYLOGENETIC ANALYSIS BASED ON SIX MENINGOCOCCAL GENES ENCODING NOVEL VACCINE CANDIDATES

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In the present study we examined the prevalence, sequence constancies and variations of the genes encoding six new meningococcal vaccine candidate genes (nmb0873-lolB, nma0939, nmb0938, nmb1796, nmb0928-nlpB, nmb0088) as well as the deduced amino acid sequences. A set of 33 Neisseria meningitidis isolates, representing clinically relevant serotypes, serosubtypes and sequence types (ST) was used. All genes were present in all N. meningitidis isolated tested. The overall mean distances, the type and number of substitutions as well as the p distances of nonsynonymous and synonymous substitutions and the pS/pN ratio for all genes are presented. A phylogenetic analysis with the neighbor-joining algorithm was performed to obtain more information about the genetic diversity of the new protein candidates. Based on the gene prevalence and conservation, five out of six antigens are promising candidates for an affective meningococcal vaccine against all N. meningitidis irrespective of serogroup. In addition, gene sequences were concatenated into a super-gene alignment, which was then analyzed to generate a phylogenetic tree. The result of this analysis was a dendogram that clusters the strains analyzed in agreement with a dendogram generated by a multilocus sequence typing (MLST) through housekeeping genes.


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Objectives: During an ongoing international effort to assess the potential coverage of the Novartis Vaccines MenB vaccine, English and Welsh ST-269 clonal complex (269cpx) isolates were genetically characterised with respect to 4 of the vaccines major components - fHBP, GNA2132, NadA and PorA. To expose underlying antigenic trends, data were profiled against Sequence Type and the genomic presence of a further marker, insertion sequence IS1301. Methods: We investigated a randomised selection of 269cpx isolates, received by the Health Protection Agency Meningococcal Reference Unit (MRU) in 2000, 2001, 2005, 2006, 2007 and 2008 (n=21, 22, 32, 41, 25 and 27, respectively). Presence and genetic diversity of gna2132, fHbp and nadA was determined using PCR and sequence analysis, and the genomic presence of IS1301 was determined using internally directed primers. Results: Only 2% of isolates possessed nadA. All isolates harboured fHbp and gna2132. The major variants, fHbp 1.11, gna2132 5, PorA P1.19-1,15-11 (and most minor subtypes), centred around ST269. fHbp 1.9-3 and 2.4, gna2132 12 and PorA P1.22-9 centred around ST275. IS1301 was present in 99% and 4% of isolates centred around ST269 and
A-10: CHAPERONINE (MSP63) COMPLEXES INDUCE BACTERICIDAL AND OPSONOPHAGOCYTIC CROSS-REACTIVE ANTIBODIES

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Introduction: Alteration of the native structure of antigens can lead to the loss of protective epitopes. Our previous results showed that separation of the meningococcal outer membrane proteins in native conditions reveal the existence of protein complexes that could be relevant for the development of new vaccine formulations. The aim of this work is the analysis of the immunogenic characteristics of a highly conserved 700 kDa chaperonin complex. Material and Methods: Separation of outer membrane protein complexes from H44/76 strain was done by high resolution Clear Native (hrCN) electrophoresis. The 700 kDa chaperonin (MSP63) complex was purified from the gel by electrolution and then used to obtain an anti-chapCx serum in mice. Characterisation of the immune response generated by the chaperonin complex was done by Western-blotting, bactericidal and opsonophagocytic assays against the homologous (H44/76) and some heterologous strains. Results: Analysis of the anti-chapCx serum by Western-blotting revealed the presence of antibodies against the MSP63 but also against the macrophage infectivity potentiator (MIP), which copurified with the chaperonin complex. This protein was shown to be highly cross-reactive with all the heterologous strains tested. Antibodies raised in anti-chapCx serum were highly bactericidal against the homologous strain and showed opsonophagocytic activity against the homologous and heterologous strains. Conclusion: Antibodies raised by immunisation with 700 kDa chaperonin complex show bactericidal and opsonophagocytic activity similar to those generated when immunisation with whole OMVs.

A-11: STUDY OF SEVERAL PRESENTATION FORMS FOR A PEPTIDE MIMETIC OF THE CAPSULAR POLYSACCHARIDE FROM NEISSERIA MENINGITIDIS SEROGROUP B

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We have selected a peptide sequence, named 4L-5, after the screening of a phage library with a monoclonal antibody directed against the capsular polysaccharide from Neisseria meningitidis [1]. In the present work, a multi antigen peptide (MAP), containing four copies of the selected peptide, was synthesized. The MAP was coupled to the carrier protein P64K using different conjugation methods. The MAP conjugated and unconjugated, was used to immunize BALB/c mice, using doses of 5, 25 and 50 ug of each immunogen. The preimmune and 4th dose sera were evaluated by ELISA using as coating antigen the unconjugated MAP and serum bactericidal activity against the homologous strain of the serogroup B strain CU385. Specific anti-peptide antibodies were elicited after immunization. The higher IgG titers were detected in the groups of mice immunized, in the three doses assayed, with the MAP conjugated to P64K using the carbodiimide method combined with previous treatment of P64K protein with succinic anhydride (MAP-CDI-Succ-P64K), with the MAP coupled to P64K using maleimide propionic acid N-hydroxysuccinimide ester as coupling agent (MAP-MPS-P64K) and in the group immunized with 50ug of the MAP containing a T-epitope from tetanus toxoid (MAP-TT). Serum bactericidal activity were evaluated in pool of sera from groups of mice immunized with the dose of 50ug. Bactericidal titers were detected in pooled sera from groups MAP-CDI-Succ-P64K (titer 1/8), MAP-MPS-P64K (titer 1/16) and MAP-TT (titer 1/32). Sera from mice immunized with these three antigens in the dose of Furthermore, sera induced against the latter antigen were able to kill N. meningitidis cells in the presence of baby rabbit complement.

A-12: SEQUENCE OPTIMIZATION OF A PEPTIDE MIMETIC OF THE CAPSULAR POLYSACCHARIDE FROM NEISSERIA MENINGITIDIS SEROGROUP B USING PEPTIDE LIBRARIES OBTAINED BY PIN TECHNOLOGY

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Conclusion: 269cpx consists of 2 broadly distinct, but well-defined lineages transcending house keeping genes and surface antigens alike, and including the presence of a specific mobile genetic element.
A peptide mimic of the capsular polysaccharide from *Neisseria meningitidis* serogroup B, named 4L-5, was identified after the screening of a phage library with the monoclonal antibody 13D9 [1]. Four amino acids of this peptide were identified as critical for mAb 13D9 binding. Sera from mice immunized with a synthetic peptide with the sequence of 4L-5 recognized not only these four residues but also an adjacent epitope containing two consecutive proline residues. Two synthetic peptide libraries, obtained by pin technology using the Fmoc/tBu strategy, were used to optimize the 4L-5 sequence in order to (i) identify peptides with higher affinity for the mAb and (ii) reduce the immune response against the undesired epitope containing the proline residues. The first 96-peptide library was synthesized introducing punctual changes in the amino acids critical for mAb 13D9 binding and substituting the stretch of two P by two A. Neither peptide containing the 2 A instead of the 2 P showed higher affinity for the mAb than the original 4L-5 peptide. We obtained a second library of 20 peptides substituting each of the P by A. In the amino acids critical for mAb 13D9 binding, were introduced the changes selected with the former 96-peptide library that influenced positively the mAb binding. Finally, three peptides were selected and synthesized as multiple antigen peptides (MAP) for further immunological studies due to their high reactivity with mAb 13D9.

**Session B: Current and New Neisserial Vaccines**

**B-1: USE OF GOLD NANOPARTICLE GLYCOCONJUGATES TO GENERATE IgG SPECIFIC FOR LACTO-N-NEOTETRAOSE**

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*Neisseria meningitidis* is the leading cause of bacterial meningitis and is a worldwide health problem. The *lgtD* gene of *N. gonorrhoeae* F62 was deleted, producing *N. gonorrhoeae* F62DlgtD. This strain produced a single lipooligosaccharide (LOS) (lacto-N-neotetraose LOS). We developed a glycoconjugate vaccine (TRIAD) that contains an oligosaccharide derived from *N. gonorrhoeae* F62??lgtD and a peptide that possesses the ability to bind to a large number of HLA class II molecules, chemically conjugated to a gold nanoparticle. TRIAD was chemically characterized and found to possess both OS and peptide on each gold nanoparticle. We were able to control the relative amount of OS and peptide on the particle by adjusting the ratio of the two conjugates used in the synthesis of TRIAD. Immunization of mice with our vaccine construct produced no observable adverse effects in C57BL6 mice. We performed as series of biweekly or triweekly immunizations. Blood was periodically collected and assayed by ELISA for the presence of antibody. Our ELISA data demonstrated that significant levels of antibody were elicited after a single immunization, with the predominant isotype being expressed being IgG; IgM levels were minimal and comparable to those elicited by LOS when used to vaccinate control groups of mice. Subsequent immunizations did not result in an increase in anti-carbohydrate titer. From these experiments, we concluded that TRIAD is capable of eliciting a potent IgG response that is directed against the lacto-N-neotetraose oligosaccharide, and that a single dose of vaccine is sufficient to generate a maximal antibody.

**B-2: METHOD OF PRODUCING MENINGOCOCCAL MENINGITIS VACCINE FOR NEISSERIA MENINGITIDIS SEROTYPES A, C, Y, AND W-135**

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Methods for producing quadrivalent meningococcal meningitis polysaccharide and conjugate vaccines for sero types A, C, Y and W-135 disclosed. *Neisseria meningitidis* fastidious medium was designed to maximize the yield of capsular polysaccharides and generate minimal cellular bio mass and endotoxin in a short duration of fermentation. The crude polysaccharides are isolated, purified and mechanically depolymerized by sonication. These purified polysaccharides were found in human clinical trials to be safe and immunogenic against meningococcal disease caused by *N. meningitidis* A, C, Y and W-135 sero groups in sub-Saharan Africa. In the preferred embodiment, the polysaccharides are conjugated to carrier proteins of diphtheria or tetanus toxoid to an average molecular size of 5100 to 9900 Daltons and provide broad spectrum protection to humans of all ages. Accelerated polysaccharide production and the efficacy of the resulting vaccine are demonstrated.
**B-3: A METHODOLOGY FOR THE PREPARATION OF IMMUNOGENIC CONJUGATES FROM NEISSERIA MENINGITIDIS CAPSULAR POLYSACCHARIDES SEROGROUPS A AND C TO TETANUS TOXOID**


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*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 their capsular polysaccharides (PsC). Five of these serogroups (A, B, C, W135 and Y) are the cause of the majority of meningococcal diseases. Conventional vaccines based on meningococcal PsC elicit an immune response in children and adults. However, their efficacy in infants and young children is limited, due to the T-independent nature of the PsC. These T-independent antigens could become T-dependent through conjugation to a carrier protein. The conjugate vaccines demonstrated to be very effective but also very expensive. Currently, the Center of Biomolecular Chemistry and the Finlay Institute have a join research project with the aim of developing conjugated vaccines against serogroups A, C and W135 of N. meningitidis. Initially a general methodology was developed for serotype C consisting of: i. Fragmentation of PsC, ii. De-O-acetylation, iii. Activation, iv. Conjugation. For that, we used acid hydrolysis, basic conditions, periodate oxidation and reductive amination respectively. The final products of these reactions were characterized by physicochemical techniques. The conjugates were immunized in Balb/C mice and the immunological response was evaluated by immunoenzymatic assays. As main results attained are high recovering in fragmentation, de-O-Acetylation and activation reactions. The conjugates have a broad and controlled carbohydrate to protein ratio and low free protein. The antibody titres elicited by the conjugates were high and specific against PsC. In summary the methodology for the N. meningitides C conjugate is ready for further development at industrial scale.

**B-4: DIFFERENTIAL ADSORPTION OF MENC/P64K CONJUGATE VACCINE CANDIDATE ONTO DIFFERENT ALUM SALTS. INFLUENCE ON THE ANTIBODY RESPONSE**

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**Introduction**: Combination vaccines are imperative to reduce the number of injections and to increase vaccination’s coverage. MenC/P64k is a polysaccharide conjugate vaccine candidate for Neisseria meningitidis serogroup C using P64k as protein carrier. A phase I trial demonstrated that MenC/P64k is well tolerated, with a good safety profile. The candidate was also highly immunogenic towards the polysaccharide component. MenC/P64k is a lyophilized formulation which is mixed with aluminium phosphate at the moment of administration. The adsorption of MenC/P64k onto alum salts has not yet been study and constitutes the aim of this work. **Materials and Methods**: Adju-Phos (2%) and Alhydrogel (3%) were diluted to 5 mg/ml in buffer phosphate. MenC/P64k was added to each gel and shaking for 5 hours at 25 °C. Samples of P64k, free oxidized polysaccharide (FPox) and P64k plus FPox were included as controls. Polysaccharide and protein adsorption were measured by the resorcinol-hydrochloride and biscinconic acid methods respectively. The resulted Alum MenC/P64k (10ìg) formulations were evaluated in New Zealand White rabbits. **Results**: Aluminum hydroxide showed high capacity (99.9%) to adsorb either MenC/P64k or P64k. This contrasted with the low capacity of Aluminium phosphate: P64k (36%) and MenC/P64k (6%). Both adjuvants showed no capacity to adsorb FPox. Rabbits immunized with MenC/P64k in aluminium hydroxide seroconvert and showed a 3 fold titer increase respect to rabbit receiving MenC/P64k in aluminium phosphate. **Conclusions**: The P64K protein mediates MenC/P64k adsorption onto Aluminium hydroxide. Data support stability studies of MenC/P64k conjugate candidate as a liquid formulation, which could facilitate combination vaccines.

**B-5: HOMOLOGOUS PRIME-BOOST STRATEGY IN ADULT MICE USING NEISSERIA MENINGITIDIS ANTIGENS AND STX1 AND STX2 TOXOIDS AS MUCOSAL ADJUVANTS**

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Both CT and the related E. coli LT are now recognized as amongst the most potent mucosal immunogens and adjuvants. The aim of this study was to determine the value of detoxified shiga toxins stx1 and stx2 (toxoids) of E. coli as a new mucosal adjuvant in adult mice. The epidemic Brasil group B meningococcal strain (B:4:P1.15,19,5,5,L3,7,9,1,8) was selected by this study and the E. coli (C7-88) O:157:H7 (Stx1) and (1189) ONT: H49 stx2+stx2vb-hb (Stx2). The formalized toxin was sampled at intervals for mouse inoculation until it became completely nontoxic for animals, Vero and HeLa cells. Balb/c received 4 doses of NOMV antigens of N. meningitidis 5 µg at 3, 7, 9, and 12 days with 2µg Stx1 or Stx2 toxoids i.n. On the 35th day, the animals were immunized i.m. with 20 µg (NOMV) and 8µg of Stx1 or Stx2 toxoids. Anti systemic antibody levels (IgG, IgM, and IgA) were measured by ELISA using native outer membrane protein of N.meningitidis as antigens. The prime-booster strategy is an effective immunization protocol for inducing humoral immune responses producing IgG antibodies of high avidity. By immunoblot major responses are elicited against class 1, 3, 4, or 5 proteins of meningococcal. Mucosal immunization has been shown to be effective for the induction of antigen-specific immune responses in both the systemic and mucosal compartments with this vaccine.

B-6: PROTEOMICS BASED ON PEPTIDE FRACTIONATION BY SDS-FREE PAGE. IDENTIFICATION OF LOW-ABUNDANCE PROTEINS FROM VA-MENGO-C-BC®

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Here we demonstrate the usefulness of peptide fractionation by SDS-free polyacrylamide gel electrophoresis and its applicability to proteomics studies. In the absence of SDS, the driving force for the electrophoretic migration toward the anode is supplied by negatively charged acidic aminoacid residues and other residues as phosphate, sulfate and sialic acid, while the resulting mobility depends on both the charge and the molecular mass of the peptides. A straightforward method was achieved for SDS-PAGE of proteins, enzyme digestion, peptide transfer and fractionation by SDS-free PAGE, which was named dual-fractionation polyacrylamide gel electrophoresis (DF-PAGE). This method increases the number of identified proteins 2.5-fold with respect to the proteins identified after direct analysis, and more than 80% of assigned peptides were found in unique SDS-free gel slices. The analysis of a membrane protein extract from Neisseria meningitidis by this approach allowed the identification of 97 proteins, including low-abundance components, 31 membrane proteins and 37 citosolic proteins. Interestingly, we identified several membrane proteins that have been evaluated by other authors as candidate for N. meningitidis type B vaccine. This is the case for NMB039431 and NMB046132 among others.

B-7: PHASE I SAFETY AND IMMUNOGENICITY STUDY OF A BRAZILIAN BIVALENT SEROGROUP B VACCINE

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Introduction: A meningococcal B vaccine candidate was produced with detoxified endotoxin (dLOS) and external membrane vesicle (OMV) to improve immunogenicity, based on the most prevalent men B strains in Brazil. Materials and methods: Open, phase I study, enrolling 30 healthy adults employed at Fiocruz. Vaccine: combined men B vaccine (MenB-Bio), with OMV of strains B:4,7:P1.19,15 and B:4,7:P1.7,1, with dLOS and Alumnum hydroxide, produced by Bio-Manguinhos, given in escalation doses of 12.5 µg, 25 µg and 50 µg (sum of both strains) in 3 doses with 2 months interval. dLOS was 1/2 dose of OMV. Clinical and laboratorial evaluation before dose 1, 48 hours after each dose, and follow up during all study. Men B bactericidal tests using human complement and avidity index (AI) done before dose 1 and 1 month after dose 3. Seroconversion (SC) defined as bactericidal titer <1/8 pre dose 1 and =1/8 post dose 3, or = 4 fold increase. Results: Safety: adverse events were mild or moderate. Larger doses were associated with higher frequency, intensity, duration and earlier appearance of adverse events. Immunogenicity: SC was, for B:4,7:P1.19,15 and B:4,7:P1.7,1, respectively: 25% and 50% (12.5µg), 66.7% and 55.6% (25 µg) and 100% and 55.6% (50 µg). There was no increase in SC for heterologous strain B:17:P1.14, although AI increased. Higher antibody concentrations pre doses 1 were inversely correlated with SC. Conclusions: MenB-Bio was well tolerated and immunogenicity is promising, allowing phase II studies.
**B-7: PHASE I SAFETY AND IMMUNOGENICITY STUDY OF A BRAZILIAN BIVALENT SEROGROUP B VACCINE**

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**Introduction:** A meningococcal B vaccine candidate was produced with detoxified endotoxin (dLOS) and external membrane vesicle (OMV) to improve immunogenicity, based on the most prevalent men B strains in Brazil. **Materials and methods:** Open, phase I study, enrolling 30 healthy adults employed at Fiocruz. Vaccine: combined men B vaccine (MenB-Bio), with OMV of strains B:4,7:P1.19,15 and B:4,7:P1.7,1, with dLOS and Alumynum hydroxide, produced by Bio-Manguinhos, given in escalation doses of 12.5 µg, 25 µg and 50µg (sum of both strains) in 3 doses with 2 months interval. dLOS was 1/2 dose of OMV. Clinical and laboratoral evaluation before dose 1, 48 hours after each dose, and follow up during all study. Men B bactericial tests using human complement and avidity index (AI) done before dose 1 and 1 month after dose 3. Seroconversion (SC) defined as bactericidal titer <1/8 pre dose 1 and =1/8 post dose 3, or = 4 fold increase. **Results:** Safety: adverse events were mild or moderate. Larger doses were associated with higher frequency, intensity, duration and earlier appearance of adverse events. Immunogenicity: SC was, for B:4,7:P1.19,15 and B:4,7:P1.7,1, respectively: 25% and 50% (12.5µg), 66.7% and 55.6% (25 µg) and 100% and 55.6% (50 µg). There was no increase in SC for heterologous strain B:17:P1.14, although AI increased. Higher antibody concentrations pre doses 1 were inversely correlated with SC. **Conclusions:** MenB-Bio was well tolerated and immunogenicity is promising, allowing phase II studies.

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**B-8: PHASE I SAFETY AND IMMUNOGENICITY STUDY OF A SEROGROUP C CONJUGATE VACCINE - LESSONS LEARNED**

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**Introduction:** Prevention of men C disease is a priority in Brazil. Local development of a conjugated vaccine is considered strategic to meet the needs of the immunization program. This is the first trial of a MenC vaccine conjugated to tetanus toxoid produced by Bio-Manguinhos, using aldehyde-hydrazide condensation chemistry (CBER/FDA). **Materials and methods:** Open, phase I study, planned to enroll 30 healthy adults employed at Fiocruz. Clinical and laboratorial evaluation were done before and 48 hours after vaccination (10mg/dose with AlOH₃). Bactericidal tests were done before vaccination and 1 month later, with human and rabbit complement. Tetanus antibodies were measured. Adverse events registered in diary cards by volunteers. **Results:** Safety: Study was interrupted after evaluation of 23 volunteers, due to more intense than expected cutaneous adverse events. Number of volunteers with adverse events was: local pain:22; edema/induration:8; erythema: 8; local pruritus: 5; erythematous plaques: 3 (1 local plaque, 1local plaque near site of vaccination and 6 plaques in abdomen, 1 local plaque with edema and warmth 9.5 x 4.5 cm); malaise: 5; cephalgia: 5; fever: 2; nausea/vomiting: 2. All 8 cutaneous events which occurred in the first 48 hours after vaccination (edema, erythema, plaques) had pre-vaccination tetanus antibody titers >8 IU/mL. There were no serious adverse events. Immunogenicity: Bactericidal titers in geometric means: hSBA: 4.38 (pre) and 6.21 (post); rSBA 16.25 (pre) and 695.37 (post); tetanus antibodies (IU/mL) 5.54 (pre) and 75.53 (post). **Conclusions:** High tetanus antibody levels before vaccination caused more intense than expected adverse events. Immunogenicity was promising.
**B-9: CHARACTERIZATION OF THE HUMORAL IMMUNE RESPONSE TO MENINGOCOCCAL PORA PROTEIN IN VOLUNTEERS IMMUNIZED WITH THE CUBAN OUTER MEMBRANE VESICLE-BASED VACCINE AGAINST NEISSERIA MENINGITIDIS SEROGROUP B DISEASE VA-MENGOC-BC®**

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This work was specifically undertaken to study the antibody response to Neisseria meningitidis PorA protein and PorA peptides in sera from 50 individuals vaccinated with the Cuban anti-meningococcal serogroup B vaccine VA-MENGOC-BC®. Specific anti-PorA antibodies were detected by ELISA and immunoblotting in sera from 56% (28/50) and 66% (33/50), respectively, of vaccinated individuals. Conformational-dependent epitopes of VR1 and non-conformational-dependent epitopes of VR2 were the main targets for the immune response whereas no response against other PorA peptides was detected. Serum bactericidal activity (SBA) was detected in the 46% (23/50) of individuals and no correlation was found between the SBA and the presence of antibodies against PorA protein. The data presented here could be useful in the analysis of the relevance of the anti-PorA antibodies in the protection afforded by this vaccine and highlights the possible importance of other antigens for the immunity conferred by VA-MENGOC-BC® against homologous and heterologous strains.

**B-10: IMMUNOLOGICAL EVALUATION IN NONHUMAN PRIMATES OF FORMULATIONS BASED ON THE CHIMERIC PROTEIN P64K-DOMAIN III OF DENGUE 2 AND TWO COMPONENTS OF NEISSERIA MENINGITIDIS**

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The main problem in the development of successful vaccines against dengue based on recombinant proteins is the necessity to use potent adjuvants to reach a proper functional immune response. Our group reported the expression, characterization and immunological evaluation of the recombinant protein PD5 in Freund’s adjuvant. This protein contains the domain III of the Envelope protein from dengue 2 virus fused to the carrier protein P64k. Therefore, to define suitable formulations for human use capable of inducing a proper functional immune response, the present work relies on the evaluation of PD5 in green monkeys [Chlorocebus aethiops sabaenus], produced with a high purity and under GMP conditions, when formulated either with outer membrane vesicles (OMV) or the serogroup A capsular polysaccharide (CPS-A) from Neisseria meningitidis. The kinetics of antibody response clearly evidenced an increase in the immunogenicity of PD5 in the formulation containing CPS-A. Coincidentally, the functionality of these antibodies was substantially high after the third and the fourth doses. Despite several reports indicating the adjuvant capacity of OMV, in this study the CPS-A exhibited a better response. For the challenge study, the immunized monkeys were inoculated with infective DEN-2 virus 45 days after the last dose. The development of viremia upon viral challenge was monitored daily by viral isolation. The virus was isolated from the sera of all monkeys in the negative control group, with a mean duration of viremia of 3.5 days. The profile obtained for the PD5-OMV group was similar to that of the negative control group, whereas in the PD5-CPS-A group, one monkey was fully protected and the other had 3 days of viremia with a low viral load (mean of this group:1.5 days). This is the first study in which the polysaccharide A of N. meningitidis is successfully employed as adjuvant for viral antigens.

**B-11: OBTENTION AND PRELIMINARY CHARACTERIZATION OF PROTEOLIPOSOMES DERIVED FROM SEROGROUP A AND W 135 NEISSERIA MENINGITIDIS**

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Serogroups A and W<sub>135</sub> of <em>Neisseria meningitidis</em> are causing recurrent meningitis epidemics mainly in Africa. Despite the use of polysaccharide (PS) vaccine, periodic epidemics occur every 8–12 years with high attack rates of 100–500/100,000. The development of a vaccine based on outer membrane protein (OMP) could be a promissory alternative. We have obtained Deoxycholate-extracted Proteliposomes (PLs) from subgroup III serogroup A and W<sub>135</sub> meningococcal strain Mk 686/02 and Mk 222/02, respectively, by the use of two different methodologies: (i) methodology used to obtain PLs from serogroup B <em>N. meningitidis</em> to produce VA-MENGOC-BC<sup>®</sup> vaccine and (ii) a new methodology was introduced in our laboratory to obtain PLs from other bacteria. PLs were analyzed by Protein and LPS determination, chromatography on Sephacryl S-1000 and SDS-PAGE Coomassie and silver stain. Using either serogroup A or serogroup W<sub>135</sub>, the purification process yield with the method (ii) was 10-times higher than method (i) and the relation Protein/LPS was similar in both cases; chromatographic profile on Sephacryl S-1000 supported the OMP-detergent assembling forming nanoparticulated structure like PL or outer membrane vesicles (OMVs); SDS-PAGE showed the presence of protein bands with described molecular weights to <em>Neisseria meningitidis</em> major outer membrane proteins, such as PorA, PorB, RmpM and OpcA, as well as small amounts of Omp85 and NspA. A PLs-based vaccine from serogroup A and W<sub>135</sub> meningococci may be an alternative to polysaccharide and conjugate polysaccharide vaccines for Africa.

B-12: NON TOXICITY FOR A SINGLE DOSE STUDY AND LOCAL TOLERANCE OF THE VACCINE AGAINST <em>NEISSERIA MENINGITIDIS</em> SEROGROUPS A AND C USING SPRAGUE DAWLEY RATS


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<em>Neisseria meningitidis</em> serogroup A is responsible for Meningitis in 60% of cases occurring at the African Meningitis Belt. Therefore, an efficient and fast vaccine against such sera-group is in great demand. vax-MEN-AC<sup>®</sup>, is a combined vaccine from sera-groups A and C meningococcal polysaccharides. In order to study the toxic potential of this vaccine, single dose and local tolerance tests were performed subcutaneously (0.5 mL) in Sprague Dawley rats. Animals were daily observed in search for local symptoms systemic toxicity signals. Food and water consumption as well as corporal weight were measured. Rats were periodically sacrificed by euthanasia to watch for possible adverse effects. During the study, neither animal deaths were reported, nor symptoms indicating toxicity. There were not differences of toxicological interest found within both experimental groups regarding the variables observed. Those results led us to conclude that under the study conditions, the vaccine candidate does not induce local adverse events, nor systemic for the animal model used after having been administered in a single dose subcutaneously, which is potentially considered as non-toxic for humans.

Session C: Neisseria Epidemiology, Pathogenesis, and Immune Response

C-1: EPIDEMIOLOGY OF MENINGOCOCCAL DISEASE IN RUSSIA

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Introduction: Meningococcal disease is still a public health problem in Russia. The notification annual incidence in different regions of country usually ranges from 1 to 3 per 100,000 populations. However, some regions may experience hyperendemic rates as high as 3 to 6 cases per 100,000 populations. The present study describes: the notification incidence of invasive meningococcal disease (IMD); the age distribution of invasive meningococcal infection; the serogroup distribution of meningococci in different regions of Russia; the mortality of invasive meningococcal infection. Aims: The epidemiology and serogroup prevalence of meningococcal infections have been studied in 47 regions of Russia. Methods: For the period 2007 the serogroup prevalence of meningococci isolated from specimens of blood or cerebrospinal fluid from children and adults was investigation. In total 601 of serogrouped cases were registered. Individual data about all cases of IMD were collected and analysed. Results: The notification annual mean of incidence in all Russia were: 2005 – 1,92; 2006 – 1,7; 2007 – 1,56 and the average for this period was 1,72 per 100,000 population. Between regions the incidence ranged widely from 0 to 6, although most regions were within the range from 0 to 3 (69 regions out of all 84 regions). Only three regions of Russia were within the range from 3 to 6 (some...
regions of Eastern and Southern parts of Russia). The highest incidence was in children under 1 year (21.4%). Over half the cases were in children under 5 years (55.2%), 13.2% of patients were in adults 15-24 years, 14.8% - were in adults 25 – 64 years and 2.57% – were in elder over 65 years . Most cases IMD in Russia were caused serogroup A (36.1%), B (24.1%), C (18.5%). There was some variation between regions of Russia. In North-Western regions nearly 46.4% of grouped cases in the period 2007 were attributed to serogroup B, 17.8% - to serogroup C and 3.6% - to serogroup A. In Central regions of Russia nearly 56.1% of grouped cases were attributed to serogroup A, 20% - to serogroup B and 17.2% - to serogroup C. In Southern regions of Russia predominated of serogroup A - 52.5%, percent of serogroup C was 19.2% and B - 12.1%. In Siberia and Eastern regions of Russia out of all grouped cases predominated serogroup B (38.5% and 50% respectively) and C (11.5% and 8.3% respectively). The average mean of mortality (overall case fatality rate - CFR) of IMD was 12.1%. Conclusion: The annual mean notification incidence in Russia were similar during the period 2005-2007 and the average was 1.72 per 100000 population. The meningococcal disease has a variation in rates of incidence and serogroup distribution around the different part of Russia. In Central and Southern regions of Russia serogroup A largely predominated. By contrast, in North-Western and Eastern regions of Russia out of all serogrouped cases IMD predominated meningococci of serogroup B.

**C-2: IMPACT OF MASS VACCINATION WITH VA-MENGOC-BC® ON NEISSERIA MENINGITIDIS POPULATION STRUCTURE IN CUBA**

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In response to epidemic levels of serogroup B meningococcal disease in Cuba during the 1980’s the VA-MENGOC-BC® vaccine was developed and introduced into the National Infant Immunization Program in 1991. Since then the incidence of meningococcal disease in Cuba has returned to the low levels recorded before the epidemic. A total of 420 Neisseria meningitidis strains isolated from patients and carriers collected between 1983 and 2005 in Cuba were analyzed by multilocus sequence typing (MLST). Among the 342 strains that were phenotypically characterized, 149 were from patients and 193 from carriers. The distribution of the serogroups was B (255 isolates), C (5 isolates), W135 (2 isolates), Z (1 isolate) and non-serogroupable (79 isolates). By MLST analysis, 63 STs were identified, 32 of which were reported as a new ST. The Cuban isolates were associated with 12 clonal complexes and the most common were the ST-32 (246 isolates), ST-53 (86 isolates) and ST-41/44 (36 isolates). This study also showed that the application of the Cuban vaccine reduced the number and frequency of the hypervirulent lineages and its impact in other lineages other than the ST-32 complex, corroborates the fact that VA-MENGOC-BC® protects some individuals against serogroup B meningococcal strains other than the vaccine type-strain. Our MLST results further demonstrated the influence of VA-MENGOC-BC® on the genetic variability of the meningococcus carriage.

**C-3: ANTIGENIC DIVERSITY AMONG STRAINS OF NEISSERIA MENINGITIDIS ISOLATED BEFORE AND AFTER MASS IMMUNIZATION IN CUBA**

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DNA sequences encoding surface-exposed VR of PorA, PorB and FetA proteins may provide important information regarding the emergence of meningococcal clones in response to population immunity. The effect of mass immunization with VA-MENGOC-BC® on the evolution of the mentioned antigens and the structure of N. meningitidis populations in Cuba were investigated. In the present study 373 strains isolated from cases and carriers that were collected between 1983 and 2005 were genetically characterized by sequencing their correspondent antigen genes porA, porB and fetA. Antigen gene sequences and MLST alleles were concatenated into unique sequences for each isolate. Phylogenetic tree inference was performed by using the maximum-likelihood method available in the PAUP* package. After sequence analysis, variants 3-1 and 3-8 are prevalent for porB; variant F5-1 was the most common for fetA, and variants 19 and 15 were prevalent on regions VR1 and VR2 of porA.
respectively. These variants match those present in the B4:P1.19,15 vaccine strain. The total of ST-32 complex isolates possessed PorB3 proteins and the most frequent combination among these clonal complex isolates was P1.19,15; F5-1. The data suggest that the ST-33, B:4:P1.19,15 epidemic strain and novel antigenic variants of that strain are responsible for the extremely low incidence of meningococcal disease that has been occurred since the introduction of the VA-MENGOC-BC® vaccine in Cuba. Evidences of occurrence of small positive selection for antigenic variation, as a mechanism of immune escape, has been found mainly in the porB antigen gene, one of the major component of Cuban vaccine.

**C-4: EPITOPE MAPPING OF ANTI-HUMAN TRANSFERRIN MONOCOCLAL ANTIBODIES: POTENTIAL USES FOR TRANSFERRIN-TRANSFERRIN RECEPTOR INTERACTION STUDIES IN BACTERIA AND HUMANS**

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Human transferrin (hTf) is a glycoprotein involved in iron transport from the absorption sites to the sites of storage and utilization that also plays a relevant role as a bacteriostatic agent preventing uncontrolled bacterial growth in the host. As an adaptative advantage, some bacteria like meningococcus, developed receptors as a mechanism for iron uptaking. To determine the number of antigenic regions recognized by a previously generated Mabs panel, we performed sequential binding experiments on Biacore. Indirect ELISA were carried out to study the reactivity of the generated anti-hTf Mabs against transferrins of different species. Epitope mapping was done using an overlapping synthetic peptide library spanning the entire sequence of the mature human protein. Additional studies to identify conformational epitopes recognized by the Mabs were performed using a random phage-display peptide library. A molecular model of the 3D structure of holo-hTf was constructed using the Modeller program to map the identified antigenic regions and corresponding hTF interacting residues. To determine the affinity of anti-hTf Mabs against apo-hTf and holo-hTf, the equilibrium dissociation constant of the interaction were estimated using surface plasmon resonance. As results, we found at least four antigenic regions in the hTf molecule. Two of the antigenic regions partially overlap with previously described transferrin-binding sites for both human receptor and bacterial receptors from two pathogenic species. Hence, such monoclonal antibodies (Mabs) could be used as an additional tool for conformational studies and/or the characterization of the interaction between hTf and both types of receptor molecules.

**C-5: MUCOSAL RECOGNITION BETWEEN NEISSERIA SPECIES AND N. MENINGITIDIS SEROGROUINS**

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Human are the only guest for *Neisseria*. The pathogenic species include *N. meningitidis* and *N. gonorrhoeae* and the non pathogenic ones are: *N. lactamica*, *N. polysachareae*, *N. Flava*, among others. *N. meningitidis* presents 13 serogroups being the most important, from the epidemic point of view: A, B, C, Y, E, and W135. They colonize the nasopharynx except *N. gonorrhoeae* that affect the genitourinary tract. The induction of systemic specific IgG that recognized until some extent different *N. meningitidis* serogroups was known. Nevertheless, the mucosal response between *N. meningitidis* serogroups and *Neisseria* species is less known. Therefore, we hypothesized that secretory (S) IgA could be induce and recognize different *N. meningitidis* serogroups and species. Human anti *N. meningitidis* Proteoliposome (PL) IgA positive saliva was selected from vaccinated young adult. Then, they were evaluated in western blotting against membrane extract (serotype antigens, STA) of mains serogroups and *Neisseria* species. The comparing of the serum specific IgG and saliva specific IgA recognition was conducted. In addition, mice were immunized nasally with AFC01 (Adjuvant Finlay Cochleate 1) to verify that recognition is not due to different circulation and that it induces mucosal response. Saliva SIgA recognizes similar proteins than serum IgG but with highest intensity. AFC01 induces, in addition to systemic specific IgG, saliva specific IgA. In summary evidences of mucosal cross-recognition among *N. meningitidis* serogroups and species of *Neisseria* are presented.
**C-6: NEISSERIA INFECTIONS IN RELATION WITH COMPLEMENT DEFICIENCY: CLINICAL PRESENTATION OF HIGH-RISK PATIENTS AND TREATMENT**

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Meningococcal disease has been reportable in Cuba since 1916. The largest Epidemic occurred in 1980 (serogrup B) 14.4X 100 mil habitant but in recent years after introduction VA-MENGOC-BC™ large epidemics have disappeared. Complement opsonizes bacteria and facilitates their uptake. It is also important for the clearance of immune complexes and apoptotic cells. Complement Deficiencies have been related with recurrent meningococcal disease or meningococcal infection with unusual serotype. We introduce results of 4 cases of Meningococcal Disease with Complement Immunodeficiency: factor C3, two of then with AR heredity. At the moment of Diagnosis the CH50 results was near zero. Two cases die with fulminant meningococcemia, one of then with diagnosis of Immune deficiency, to late for impose treatment do to rapid progress (48 hours) and in the other one was knower the state of Immunodeficiency but the patient assist to late at urgency. The others two cases survive to Meningococcal using IG treatment, plasma, antibiotics and general measurement of support. Is interesting that 3 of 4 patients were female and one male that also suffer infection with *Neisseria gonorrhoeae*. Therefore, the most effective strategies to prevent meningococcal disease include identification of high-risk groups taking also in consideration Complement Immunodeficiency.

**C-7: INTRATHECAL SYNTHESIS OF IMMUNOGLOBULINS AND C3C IN BACTERIAL MENINGOENCEPHALITIS**

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Bacterial meningoencephalitis constitutes an important source of morbidity, mortality and disabilities in different regions of the world. The objective of this paper is to know if complement system can be involved in producing- meningoencephalitis bacterial lysis through C3c release into cerebrospinal fluid. Seven patients were studied with age 3 year-old average that attends Pediatric Hospital of San Miguel del Padrón by lumbar punction diagnosis. It has been isolated the following germs: *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. The quantification of C3c, albumin and main immunoglobulins in serum and cerebrospinal fluid were carried out by radial immunodiffusion in commercial plates. Results: One of the two patients who died had no detectable C3c in both biological fluids. Intrathecal synthesis of C3c was observed in the other ones demonstrated by the corresponding reibergram specially designed for this component. Conclusions: Intrathecal synthesis of this component of complement system is useful to discriminate immunodeficiency and to understand the behaviour of this disease.

**C-8: C3C INTRATHECAL SYNTHESIS IN MENINGOENCEPHALITIS DUE TO NEISSERIA MENINGITIDIS**

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*Neisseria meningitidis* meningoencephalitis had strongly affected Cuban children population before the Cuban vaccine campaign. The aim of the study is to evaluate the complement system role in the development of the disease in non-vaccinated children suffering from this disease. Patients and methods: Seven children 5.8 years old average with *N. meningitidis* meningoencephalitis was diagnosed by the traditional microbiological cultures. Sera and cerebrospinal fluid samples were simultaneously obtained and measured C3c, major immunoglobulins and albumin by radial immunodiffusion in commercial plates. Results: One of the two patients who died had no detectable C3c in both biological fluids. Intrathecal synthesis of C3c was observed in the other ones demonstrated by the corresponding reibergram specially designed for this component. Conclusions: Intrathecal synthesis of this component of complement system is useful to discriminate immunodeficiency and to understand the behaviour of this disease.
**C-9: MICE IMMUNE RESPONSE TO BRAZILIAN DTP-HIB COMERCIAL VACCINE COMBINED WITH EXPERIMENTAL MENINGOCOCCAL B AND C VACCINES**

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The Brazilian Ministry of Health has indicated an increasing interest in combined vaccines to be used in the National Program of Immunization (NPI). This approach may reduce costs by improving logistics of mass vaccination campaigns in general. Therefore, we combined two experimental vaccines against Neisseria meningitidis, based on outer membrane vesicles (OMV) for group B and conjugated vaccine for group C meningococci, both of which are currently undergoing phase II and I clinical trials, respectively, with the licensed DTP-Hib vaccine in use routinely by the Brazilian NPI. The immune response elicited by the combined vaccine was evaluated in mice immunized with three doses of DTP-Hib/B/C vaccine. The total IgG to each antigen was measured by ELISA and the data indicates no interference among the antigens with regard to antibody titers. A 4-fold or higher increase in antibody titers was observed for all antigens after 30 days of the last doses. Ongoing work includes reducing the number of doses required for effective immunization. This work is the basis for the development of a hexavalent bacterial vaccine to be used in the Brazilian setting.

**C-10: MOLECULAR UPDATE OF THE EPIDEMIOLOGY OF SEROGROUP Y MENINGOCOCCAL DISEASE IN LATIN AMERICA**

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**Introduction:** Within the context of SIREVA II, a Latin-American network focused in bacterial meningitis, an increase in serogroup Y invasive meningococcal cases has been notified in Colombia since 2004, ranging between 30 to 50% of the cases during the last years. Unstable features have been notice in Argentina and Costa Rica, with ups and downs in the number of serogroup Y cases. By contrast, group Y meningococci are only sporadically isolated in other countries at the same geographical area like Brazil and Chile. **Aims:** In order to improve the knowledge of the serogroup Y MD in Latin-American region, we analyzed group Y strains received in the National Institutions of these countries over an 8 years period (2000-2007). **Material and Methods:** One hundred and eighty four group Y isolates (65, 49, 19, 40 and 11 strains isolated in Argentina, Brazil, Chile, Colombia and Costa Rica respectively) were characterized. Serotyping and serosubtyping were done by whole-cell. The genotype defined by porB and/or porA sequencing was done in non-serotypable (NT) and/or non-serosubtypable (NST) strains, but also in a random sample of typable strains. Sequence type, clonal complex and fetA allele designation was done according to the Neisseria MLST website (http://pubmlst.org/neisseria/). **Results:** The serogroup Y distribution from 2000 through 2008 as determined by the National Laboratories is presented in Figure 1. Thirty six STs were found but most of the strains appeared to be distributed in only 3 groups: 2 defined by clonal complexes (ST-23 CC and ST-167 CC) and the other one by ST5770 (without CC assigned) and STs representing single or locus double variants of ST5770. An apparent association between the antigens expressed by a strain and its ST was observed regardless of increase number of cases: isolates belonging to the ST-23 CC were mainly characterized as 14:NST (PorA 5-2,10-1); most of the strains from ST-167 CC were NT (porB 3.95 (4d.D.7.14a)):P1.5; and the ST-5770 and related STs were frequently associated with NT (porB 3.100(B.C.7.14b)):P1.5. Two differentiated situations were notice: the first one, observed only in countries with increase of serogroup Y cases (Argentina, Colombia and Costa Rica), with a predominant clone all over the period (CC-23 in Colombia and Costa Rica and CC ST-167 in Argentina); and the second one, experiencing low-level and stable serogroup Y distributions without a dominant clone (Brazil and Chile). **Conclusion:** The situation observed in Colombia but also in Costa Rica is similar to that happen in United States, particularly in the prevalence of STs belonging to the CC ST23. It is likely that the situation in Argentina is result of an independent event, with data suggesting a probable epidemic wave with a particular ST predominating. Our data appear to document 2 instances, but other instances may fall the level of detection of the SIREVA II network. Going forward, future surveillance data may be able to evaluate the use of new conjugated meningococcal vaccines in the region and influences emanating from nearby countries.
C-11: ASSESSMENT OF THE IMMUNE RESPONSE INDUCED BY PROTEO LIPOSOMES DERIVED FROM NEISSERIA MENINGITIDIS SEROGROUPS A AND W135

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Neisseria meningitidis serogroups A and W 135 are the main causative agents of recurrent epidemics in the so called Meningitis Belt in Africa. Though polysaccharide vaccines have been used in this region, periodic epidemics occur every 8 to 12 years (100 to 500 cases/100 000 inhabitants). The search of more effective vaccinal candidates leads to the development of plain or combined outer membrane protein polysaccharide vaccine as a promising alternative. We have obtained proteoliposomes (PLs) extracted from the outer surface of A and W 135 strains (Mk 686/02 and Mk 222/02, respectively) using Sodium deoxicolate. Two different methods were used: (i) the one used in the manufacturing process of PL of VA-MENGOC BC® vaccine, (ii) a new method developed in our laboratory to obtain PL derived from other bacteria. Obtained PL for each strain type was administered to Balb/c mice by intraperitoneal route. Twenty-one days after immunization they were bled to assess the systemic IgG anti-PL immune response by ELISA. Sera of highly respondent mice were mixed and used in Western blot assays in order to assess reactivity to cellular lisates (A and W135 serogroup strains) and to the corresponding PLs. ELISA assessment showed a high antibody response (without significant differences) for PLs derived from both A and W135 strains, no matter which method was used for purification. Western blot assay showed a high homologous reactivity (mainly with protein bands of molecular weights similar to those described for Omp85, PorA, PorB, RmpM and NspA), and a weak heterologous reactivity (mainly for PorA and PorB). Though these results are preliminary, they show the potentiality of PLs derived from A and W135, as alternative vaccinal candidates for current polysaccharide vaccines.

C-12: NASAL IMMUNIZATION WITH AFCo1 INDUCES IMMUNE RESPONSE TO N. GONORRHOEA IN MICE

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Neisseria gonorrhoeae infections are common sexually transmitted diseases. Increased antibiotic-resistant of N. gonorrhoeae strains were reported. N. meningitidis is another human restricted bacterium transmitted through mucosa. Although the induction of systemic specific IgG antibody against some proteins between the two species is known, the mucosal immune response to these pathogens is not clear. We hypothesized that N. meningitidis could induce cross-reactive immune response against N. gonorrhoeae. To test this, serogroup B Proteoliposome (PL) was transformed into AFCo1 (Adjuvant Finlay Cochleate 1) and used for nasal immunization of C57Bl/6 mice. The specific IgG and IgG subclasses against both antigens in sera and vaginal extraction were measured by ELISA. Specific proliferation (3H incorporation) of spleen cells and lymph node recall in vitro with PL or N. gonorrhoeae total antigens was measured. Serum and vaginal extraction anti N. meningitidis and N. gonorrhoeae IgG as well as the induction of specific IgG subclasses were detected. N. gonorrhoeae induces specific proliferation of spleen, cervical lymph node (cLN), and mediastinal (meLN) cells from immunized mice. In conclusion, nasal immunization of mice with AFCo1 induces anti N. meningitidis immune responses that could recognize N. gonorrhoeae antigens.

C-13: MUCOSAL AND SYSTEMIC IMMUNE RESPONSE AGAINST NEISSERIA MENINGITIDIS B INDUCED BY SINGLE TIME VACCINATION STRATEGY

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Vaccination is considered by the WHO to be the most cost-effective strategy for controlling infectious diseases, but a successful programme of immunization can contribute much more than just vaccines. Epidemiologically targeted implementation of vaccines has diminished morbidity and mortality from many infectious diseases that previously were scourges and economic burdens (such as measles, polio, diphtheria, Haemophilus influenzae type b, meningococcal, and pneumococcal infections). However, immunization remains an unfinished agenda, because globally and in some regions immunization coverage has increased only marginally, being still inefficient. Several factors have been largely responsible of a difficulty to attain immunization coverage and have been recognized as a problems of current vaccines, such as: the number of doses, excessive use of parenteral route over mucosal route, inadequate progress in the field of vaccine adjuvants for human use, high reactogenicity, unavailability against intracellular pathogens, infected or altered cells, and scanty feasibility to combined more than one antigen in the same formulation and others. In meningococcal disease, the assumption that currently available vaccines are strain specific is another issue. In addition, current vaccines are parenteral protecting against the disease but not totally eliminated the carriage stage, cause of disease transmission. Therefore, Single Time Vaccination Strategy (SinTimVaS) was developing. SinTimVaS induces in a single time vaccination contact systemic and mucosal response. The use of nasal AFCo1 and parenteral AFPL1 at the same time induces serum anti PL IgG and mucosal anti PL IgA. The function of SinTimVaS was not exclusively of these adjuvants. The induction of specific IgA was observed in regional and distal places. SinTimVaS also function by other mucosal routes like, oral and sublingual. In conclusion a new proprietary strategy was developed which could influence the time, costing and coverage of vaccination.

C-14: NASOPHARYNGEAL CARRIERS OF NEISSERIA MENINGITIDIS IN A GROUP OF WORKERS WITH OCCUPATIONAL RISK

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The carriers of Neisseria meningitidis constitute the main source of infection and transmission of the Meningococcal Disease (MD). To know their prevalence, the characteristics of the isolated strains and the factors of risks associated with the carrier state, contribute to data of extraordinary value for the control and monitoring epidemiologist of this clinical organization. In order to compliment the proposed objectives a descriptive observational study of carriers of N. meningitidis was made in 112 individuals with ages between 18 - 60 years in a Center of Biopharmaceutical Production of City of Havana. Previous to its accomplishment it was fulfilled the required bioethics exigencies for this type of investigation. To all the participants an exudate was taken from nasopharyngeal later and a survey was made to them where it was investigated about the possible factors of risk (age, sex, habit to smoke, consumption of spirits, antecedent of respiratory infection) which they favour the condition of the carrier. The identification of the stocks of N. meningitidis isolated was made according to the conventional methods and the classification of the serogroups became by lamina agglutination with specific commercial antiserums serogroup. Whereas, for the classification of serotypes and subtypes a immunoenzimatic test (ELISA) of whole cells with monoclonal antibodies (AcM) was used. A 8% of carriers of N. meningitidis were detected, with predominance of serogroup B (77.8%) and the more frequent phenotypic association was the B: 4: P1.4 (33.3%). When analyzing the state of carrier and its possible association with the risk factors were observed that no influenced of statistically significant form with this condition. This work demonstrated the possibility of the occupational risk in those individuals that by their profession are in contact with the enemy with pathogenic micro organisms. The (ELISA) of whole cells with (AcM) was used. An 8% of carriers of N. meningitidis were detected, with predominance of serogroup B (77.8%) and the more frequent phenotypic association was the B: 4: P1.4 (33.3%). When analyzing the state of carrier and its possible association with the risk factors were observed that no influenced of statistically significant form with this condition. This work demonstrated the possibility of the occupational risk in those individuals that by their profession are in contact with the enemy with pathogenic micro organisms.

C-15: INTRANASAL IMMUNIZATION WITH AFCO1 INDUCE SYSTEMIC, MUCOSAL AND MEMORY IMMUNE RESPONSE IN NEONATAL MICE


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Neonates have a poorly developed immune system. Respiratory pathogens cause disease during early periods of life. Consequently, it is important to develop protective vaccines that induce immunity and immunological memory against respiratory pathogens early in life. Intranasal (i.n.) route could be an effective via for immunization. Therefore, we explored the effectiveness of AF (Adjuvant Finlay) PL1 (Proteoliposome) from Neisseria meningitidis serogroup B and its derivate Cochleate (AFCo1) by nasal route in neonatal mice. They were immunized i.n. 3 times 7 days apart and anti PL systemic and mucosal antibody
response were measured by ELISA. In addition, a prime-boost strategy was used to evaluate the humoral immune response in neonate mice. The 3 doses of AFPL1 or AFC01 induced significant levels of anti PL IgG antibodies in comparison with control, but AFC01 (2017 U/mL) was significantly higher than AFPL1 (1107 U/mL). AFC01 and AFPL1 induced a predominant Th1 pattern with IgG2a/IgG1 > 1 by i.n. immunization and AFC01 induced a high anti PL IgA saliva response in saliva. Interestingly, one nasally prime at 7 days of born and a memory one boost i.n. dose 9 weeks later with AFC01 or AFPL1 showed similar specific IgG levels and IgG2a/IgG1 relation than 3 i.n. doses in adult mice. In conclusion, these results represent the first report of neonatal intranasal vaccination using AFC01 capable to induce systemic and mucosal immunity and priming for memory.

**D-1: STUDY OF THE INFANT RAT PASSIVE PROTECTION ASSAY CONDITIONS TO EVALUATE MENINGOCOCCAL VACCINE CANDIDATES BASED ON SUB-CAPSULAR AND POLYSACCHARIDE ANTIGENS**


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_Neisseria meningitidis_, the etiological agent of meningococcal meningitis and meningococcemia, is still an important cause of mortality throughout the world. The bacterial virulence and colonization strategies are highly adapted to humans, being difficult to study it in animal models. Meningococcal host specificity is due in part to the specificity of the iron-acquisition receptors on the surface of the bacteria, because the transferrin and lactoferrin receptors are specific for the corresponding human substrates. In an attempt to substantiate data obtained from in vitro assays, researchers have employed animal models that mimic human disease. Meningococcal disease has been modeled with infant mice and rats, since infant rodents, are more susceptible to meningococci. In our study, the neonate rat infection model, developed in the 80’s at the NPHI, Helsinki, Finland, was improved to consistently give reproducible results in terms of bacterial counts in blood of infant rats intraperitoneally challenged with some meningococcal strains. Factors like animal age, weight, target strain growth conditions and rat passages, challenge dose and the coadministration of a parenteral dosage of iron dextran as enhancer were considered. The adapted model has been successfully used to study the protection afforded by passively transferred antibodies, to identify antigens involved in protection and also to test the protective activity of candidate vaccines based either in capsular or sub-capsular antigens. Finally, at present this animal model is reproducible for some serogroup B and C strains. However, other strains need careful choice of the assay conditions for sensitivity and repeatability.

**D-2: ASSESSMENT OF WORKING CONCENTRATION OF BACTERIA TO BE USED IN THE WHOLE BLOOD ASSAY**

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The Serum Bactericidal Assay (SBA) has been considered the “golden standard” to evaluate the serological efficacy of meningococcal vaccines, taking into account that the presence of serum bactericidal antibodies is related with protection. The levels of A and C capsular polysaccharide antibodies against these serogroups had confirmed the reliability of this assay. The SBA has also been used to reassess the efficacy of serogroup B vaccines, but in some studies, the correlation between serological efficacy and protection has been poor. Ison et al developed the Whole Blood Assay (WBA) that measures the complete bactericidal activity. The results obtained indicate that this model is a more sensitive marker of immunity that SBA for serogroup B. The results from the evaluation of Cuban meningococcal vaccine (VA-MENGOC-BC®) in infants using WBA showed after the immunisation around 50% of infants exhibited >50% killing of the vaccine strain (B:4:P1.19,15). The results against heterologous strains were poor. Taking into account these and other results we evaluated the behavior of the WBA using lower concentration of the bacterial suspension as inoculum. Blood from two healthy adults was screened for bactericidal activity using the WBA, against B:4:P1.19,15(Cu 385/83) and B:15:P1.7,16 (MC58) strains. In addition to the recommended concentration (10⁶ or 10⁷ CFU/mL), two lower concentrations were used (10⁵ and 10⁴ CFU/mL). The first donor showed a lactic activity of 40% against homologous strain, at 10⁷ CFU/mL. At 10⁶ and 10⁵ CFU/mL the lisis was around 80%. The behavior against heterologous strain was 25% of lisis at 10⁷ CFU/mL and around 60% at lower concentrations. The second donor did not show lactic activity against 10⁷ CFU/mL of homologous strain, while it obtained 55% and 65% at 10⁶ and 10⁵ CFU/mL respectively. The results against heterologous strain were 40% of lisis at the highest concentration and 70% and 80% at 10⁶ and 10⁵ CFU/mL respectively.
**D-3: CUBAN EXPERIENCE AND WORLDWIDE STATE OF THE ART IN THE DEVELOPMENT AND PRECLINICAL ASSESSMENT OF MENINGOCOCCAL VACCINES**

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Infectious diseases experimental biomodels has been utilized successfully during the development and preclinical evaluations of experimental or investigational meningococcal BC vaccines due to the impossibility of obtain consistent correlates of protection by means of *in vitro* techniques. Only in experimental biomodels can be evaluated the interaction of this microorganism with the innate, humoral or cellular immune system responses. Nevertheless, only the humans are the natural hosts for *Neisseria meningitidis* and there are not a perfect laboratory animal disease model that represent properly the clinical features of the human disease. Two main animal models have been comprehensively and consistently utilized: a) the adult mice intraperitoneal infections; and b) the infant rats. The first one need a exogenic iron source, as the human transferrin, in order to obtain a lethal bacteraemic infections capable to evaluate both active and passive immunisation; the second model (infant rats) has been used to evaluate passive protection by means sera raised against vaccine candidates or human vaccine sera. Unfortunately, in both models the bacteraemia is short, mortality is low and is not possible evaluate the active protection. Recently, has been developed other hopeful approaches, i.e. transgenic mice biomodels expressing CD46, wild types and knock out mutants. We are presenting here our 15 years experience at the Finlay Institute, in the preclinical evaluation and assessment of developmental and investigational meningococcal BC vaccines in Cuba and another vaccine candidates, by means of experimental biomodels.

**D-4: OPSONOPHAGOCYTIC AND C3C COMPLEMENT DEPOSITION RESPONSES ELICITED IN ADULT VOLUNTEERS AFTER THREE OR FOUR DOSES OF THE MENINGOCOCCAL SEROGROUP B OUTER MEMBRANE VESICLE VACCINE MENZB**


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MeNZB is a meningococcal serogroup B outer membrane vesicle vaccine which was developed in response to a clonal epidemic of B:4:P1.7-2,4 in New Zealand. A phase II study in the UK compared the immunogenicity of a three dose (0, 12 and 60 weeks) or four dose (0, 6, 12, and 60 weeks) vaccination schedule. Fifty volunteers aged between 18 and 40 were vaccinated and had blood samples taken at 0, 6, 12, 15, 60 and 63 weeks. In this study the antibody-mediated opsonophagocytic and complement deposition responses stimulated by the vaccine have been determined. The antibody-mediated opsonophagocytic assay (OPA) was performed using HL60 granulocytic cell line differentiated with DMF, azide-fixed *N. meningitidis* labelled internally with BCECF, and IgG-depleted baby rabbit complement. The complement deposition assay (CDA) was performed using fixed, unstained *N. meningitidis*, IgG-depleted human plasma, and fluorescent anti-human C3c antibodies. Both assays were performed against the homologous strain of MeNZB (NZ98/254), H44/76-SL, and 5 strains isolated in the UK. A Z-test was used to identify individuals who showed a significant rise in OPA or CDA activity from 0 to 15 or from 0 to 63 weeks. MeNZB elicited a strong response against the homologous strain with over 80% of subjects showing a significant increase in OPA and CDA activity after four doses. A large percentage of subjects receiving both the three and four dose schedules also showed a significant rise in opsonophagocytic activity and complement deposition activity against the six heterologous strains, depending on strain.

**D-5: CONSISTENCY APPROACH: A SUITABLE ALTERNATIVE FOR THE QUALITY CONTROL OF MENINGOCOCCAL B-C VACCINE BATCHES?**

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The disadvantages of *in vivo* tests are numerous: they are expensive, inaccurate, slow and ethically questionable. Nevertheless, for many vaccines in *in vivo* potency testing is performed on each batch. As a result, lot release testing of vaccines requires substantial number of laboratory animals of various species. For more recently developed vaccines, a better handle on consistency in production through in-process testing has allowed simplification of release protocols, in particular by using *in vitro* tests,
whilst maintaining the capacity to demonstrate equivalency with clinical lots. Despite of the limitations for some adsorbed vaccines that have already been on the market for a long time, a consistency approach can be implemented, thus reducing the need for extensive final lot testing, often associated with the use of animals. This new approach must build on information obtained during product development (including clinical testing) and routine production using suitable analytical tools and trend analysis. The present work aims to provide an updated overview on the state-of-the-art of the consistency approach for vaccines as well as to propose a strategy for its implementation as part of quality control of Meningococcal B-C vaccine batches. Until now the biological assays have been determinant for lot release of this kind of product, nevertheless its role could be reduced if meaningful physico-chemical, biochemical and in vitro tests are undertaken to define a product profile focused on demonstrating equivalency with clinical lots.

**D-6: ANALYTICAL EVALUATION OF SEVERAL INTERMEDIATES AND THE FINAL MONOVALENT CONJUGATES FROM N. MENINGITIDIS SEROGROUP A AND C-TETANUS TOXOID**

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Bacterial meningitis and Meningococcal septicemia constitutes a significant global health problem. In particular, *Neisseria meningitidis* (Nm) is responsible of a significant global health problem and continues to be a public health problem among human populations. Specially A, B, C, W135 and Y serogroups are frequently source of pneumonias and otitis media in children less of five year old. For this reason our project involves new conjugate vaccine from capsular polysaccharides A and C to Tetanus Toxoid using different methods. In order to evaluate the modified polysaccharides during the process and the final conjugates we employed the following methods: a) colorimetric such as: Chen, Hestrin, Lowry and Svennerholm; b) HPLC with Superose 12 and TSK5000PW columns; and c) ¹H NMR experiments. The identity analysis was performed using ¹H NMR spectroscopy as well as the O-Acetylation that was compared by the former methods and by colorimetry using the Hestrin procedure. An important issue is quantification of the polysaccharide that was established for C serogroup by Svennerholm colorimetric methods and by HPSEC with Superose 12. For A serogroup the quantification of the polysaccharide content was developed using TSK5000PW and using the Chen method as complementary technique. The quantification of conjugate was assisted with the Lowry procedure. Finally Conjugates and polysaccharides from serogroups A and C were evaluated combining different colorimetical, chromatographic and spectroscopic methods.

**D-7: IDENTIFICATION AND QUANTIFICATION OF FATTY ACIDS IN LIPOOLIGOSACCHARIDE OF NEISSERIA MENINGITIDIS SEROGROUP B BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY**

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The meningococcal disease remains a world wide health problem. Brazil has been a public health problem since serogroup A and C epidemics occurred in 1970’s. From 1990 to 2000, the annual incidence in Brazil ranged from 1 to 3 per 100-000 inhabitants. Serogroup B presently accounts for 60% of clinical isolates from meningococcal infections, followed by 40% due to serogroup C. Bio-Manguinhos/Fiocruz, a Brazilian vaccine producer, has been involved on development of a meningococcal B tailor made vaccine made by OMV and detoxified LOS (dLOS) from prevalent strains. The dLOS inclusion as a vaccine antigen seems to be an innovation considering the similar vaccines that have already developed all over the world. This study has been conducted to identify the LOS components before and after the detoxification procedure to ensure the production steps of the immunogen. The vaccine antigens have been obtained from bacteria biomass and the purified LOS has been detoxified in basic conditions. Four representative fatty acids resulting from the basic hydrolysis of the LOS lipid portion have been identified and quantified using GC-MS. The lauric acid represents around 70% of these total fatty acids generated. The LOS structure has been compared before and after the detoxification procedure by NMR ¹H and ¹³C proving a significant lipid aliphatic portion reduction. The successful results on the LOS detoxification and characterization/ quantification of fatty acids are extremely relevant for encouraging the validation of this analytical methodology and development of a quality control for this vaccine production.
**D-9: BACTERICIDAL ACTIVITY IN MICE OF AN OUTER MEMBRANE PROTEIN VESICLE AGAINST NEISSERIA MENINGITIDIS SEROGROUPS A AND W**

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**Neisseria meningitidis** remains as one of the leading infectious causes of death of devastating epidemics in nonindustrialized nations. The protective role of antibodies able to promote complement-mediated killing of bacteria (bactericidal activity) was demonstrated in the 1960s and this assay is used currently for evaluating vaccines against serogroups A, C, Y and W<sub>135</sub>. Polysaccharides vaccines are still used only in people at increased risk. They have never been considered for universal vaccination because of several shortcomings that compromise their utility. The outer membrane proteins have been extensively studied as potential vaccine constituents. Evidences suggest that a certain titer of bactericidal antibody might be correlated with protection. Because of these difficulties in realizing an effective polysaccharide vaccine, researchers have turned their attention to other bacterial surface structures as outer membrane vesicles. In this paper the immunogenicity of outer membrane vesicles purified from 806/03 (serogroup A) and 222/02 (serogroup W<sub>135</sub>) strains was evaluated in mice by bactericidal assay using as antigen F8238, 686/02, 499/03, 806/03 (serogroup A) and 196/02 and 222/02 (serogroup W<sub>135</sub>) strains. Baby rabbit serum was used complement source. The bactericidal titer for each unknown serum is expressed as the reciprocal serum dilution yielding ±50% killing as compared to the number of target cells presents before incubation with serum and complement. Sera from mice immunized resulted in significant levels of bactericidal activity towards target strain from homologous serotype used in the assays. None of the control sera showed bactericidal activity towards F8238 and 222/02 strains.

**E-1: SUSTITUTION OF SOLID MEDIUM IN THE TECHNOLOGY FOR OBTAINING CULTURE OF NEISSERIA MENINGITIDIS TO PRODUCE PURIFIED CAPSULAR POLYSACCHARIDE**


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The process of obtaining inoculum for microbial fermentation in the production of vaccines, is the starting point to obtain a good growth with an adequate antigenic expressions acceptable of the microorganisms, also the use of the medium with a composition...
defined supporting cultivations as much liquids as solids, is also primordial to obtain a good recovery of the microorganisms. The present work has as objective to evaluate the productive effect in the substitution of the culture solid medium in the obtaining of cultivations for the production of purified capsular polysaccharide of Neisseria meningitidis. In this study evaluated the kinetic behavior of growth through direct liquid passes from seed bank stored to -70°C. For the assays was employed flask with 200ml of culture medium in shaker with 200 rpm stirring determining the absorbance every hour and viability at the beginning and end of the culture. The results were comparable in terms of productivity or promotion growth with the traditional culture medium. It was demonstrated that the substitution of the culture medium do not have influence in the productive consistent. Also the conditions and time of the process was reduced without afectation of the antigenic expressions.

**E-3: DETERGENT EFFECT IN MENINGOCOCCAL OUTER MEMBRANE COMPLEXES ANALYSIS BY HIGH RESOLUTION NATIVE ELECTROPHORESIS**

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We are leaving in a complex world with thousands of problems with out any solution yet. Some of them are related with the complicated situation with the health in underdeveloped countries surrounded by dangerous diseases that are nowadays serious epidemics: AIDS, TB, Malaria, tropical diseases as dengue, yellow fever, typhoid fever, and others. Economical disparity between the most developed countries and poor countries, climatic and natural disasters, military conflicts, political incapacity and others causes have provoked a worrying reality that contribute with millions of deaths yearly. The lack of medicaments, vaccines and drugs against those diseases and the assumed position by biopharmaceutical companies and others global institutions which are most interested in their own profits what the benefits that any vaccine or medicament could result for the human been, are situations what testify how at the 21st century the underdeveloped peoples don’t have the minimum health conditions to survive. With this article we wish to characterize the main protagonist of the biotechnology field, mainly related with Vaccines topic, using the situation of the meningitis disease as main focus. What are doing the companies, governments, NGO and other institutions in order to give solutions to epidemic crisis in underdeveloped countries lashed by meningitis epidemic? We are going to remark during the exposition the meningitis situation. We will demonstrate that with the combination of some aspects as good political intentions, a serious scientific work and other necessary strengths, humanity could be the winner in the war against meningitis.

**E-2: THE VACCINE GLOBAL MARKET AND ITS POLITICAL INFLUENCE IN UNDERDEVELOPED COUNTRIES**

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We are leaving in a complex world with thousands of problems with out any solution yet. Some of them are related with the complicated situation with the health in underdeveloped countries surrounded by dangerous diseases that are nowadays serious epidemics: AIDS, TB, Malaria, tropical diseases as dengue, yellow fever, typhoid fever, and others. Economical disparity between the most developed countries and poor countries, climatic and natural disasters, military conflicts, political incapacity and others causes have provoked a worrying reality that contribute with millions of deaths yearly. The lack of medicaments, vaccines and drugs against those diseases and the assumed position by biopharmaceutical companies and others global institutions which are most interested in their own profits what the benefits that any vaccine or medicament could result for the human been, are situations what testify how at the 21st century the underdeveloped peoples don’t have the minimum health conditions to survive. With this article we wish to characterize the main protagonist of the biotechnology field, mainly related with Vaccines topic, using the situation of the meningitis disease as main focus. What are doing the companies, governments, NGO and other institutions in order to give solutions to epidemic crisis in underdeveloped countries lashed by meningitis epidemic? We are going to remark during the exposition the meningitis situation. We will demonstrate that with the combination of some aspects as good political intentions, a serious scientific work and other necessary strengths, humanity could be the winner in the war against meningitis.