REPORT

Recent Advances in the Pathogenic Neisseria Research

Selected topics presented at the XVI International Pathogenic Neisseria Conference (7-12 September 2008, Rotterdam, Netherlands)

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Introduction

Neisseria meningitidis, a Gram-negative bacteria, is an important cause of bacterial meningitis and septicemia worldwide [1]. Meningococcal disease is a life-threatening illness that may progress to death even after medical intervention. Despite treatment with appropriate antibiotic therapy, the case fatality rate remains high, and survivors can have significant sequelae, including neurological disability, limb loss and hearing loss. The highest incidence of the disease occurs in infancy due to the lack of protective antibodies [2].

Five defined serogroups (A, B, C, W-135 and Y), based on distinctive capsular polysaccharides (CPS), cause the most cases of meningococcal disease globally. Prevention through vaccination remains the most effective approach to control invasive meningococcal disease. Vaccines are available for preventing meningococcal disease caused by serogroups A, C, W-135 and Y, but, to date there is no effective vaccine against disease caused by a broad range of serogroup B strains [1].

The nature of the serogroup B CPS, which is poorly immunogenic and shares some antigenic determinants with human tissues [3], has complicated the development of a suitable universal vaccine aimed to induce antibodies that target the polysaccharide capsule, which is, by definition, the most conserved antigen within the serogroup.

For serogroup B, non-capsular approaches are being pursued, including those that target antigens contained in the outer membrane vesicles (OMV) produced by the bacteria [4, 5]. However, outer membrane structures may vary between strains, and as a result OMV-based vaccines induce immune response mainly protective against the original strain. These types of vaccines have been successfully used in controlling the disease in specific regions where the circulating strains are identical or very similar to that used to produce the vaccine.

To overcome antigenic variability most vaccine developers have sought highly conserved surface antigens, which will confer a cross-reactive immune response The identification of this kind of antigens is a promising strategy to develop a truly universal vaccine against serogroup B meningococcal disease. These antigens, present in a high number of strains and antigenically conserved could be used as stand alone vaccines or combined with OMV-based vaccines to increase the OMV range of protection.

New approaches combining conventional vaccine strategies with genome-derived technology, with the availability of the meningococcal sequenced genomes, proteomics and other [2], have accelerated the process of identification and testing of new vaccine candidates and have brought new potential vaccine candidates to preclinical and clinical trials.

Due to the worldwide importance of the meningococcal disease, in the 1970´s a series of conferences were held dealing with issues of meningococcal epidemiology and vaccination. Some of these conferences were held in Milano, St Paul de Vence and Marseille. However, the first official Neisseria Conference was held in San Francisco, California in 1978. Since then a Conference is held every two years to discuss the new advances in the Neisseria field ranging from genetics, surface structures, pathogenic mechanisms, and host interactions, to epidemiology and vaccine development.

The XVI edition, held in Rotterdam, Netherlands, from 7 to 12 of September, 2008, gathered 491 delegates from 41 countries [data from the Congress Organi-zers]. The conference consisted in more than 60 plenary lectures and selected oral presentations and 240 posters from submitted abstracts with plenty time for discussions. The presentations were organized in the following symposia: 1) Bacterial genetics, physiology and metabolism; 2) Host and pathogen genomics and gene expression; 3) Antibiotic resistance; 4) Host response, immunology and experimental therapy; 5) Vaccinology (preclinical); 6) Epidemiology; 7) Cellular microbiology; 8) Surface structures and 9) Vaccinology (clinical). There was also a session to discuss the Novartis investigational meningococcal B vaccine, two parallel sessions dedicated to The Meningitis Vaccine Project and The International Collaboration on Gonococci and a Poster Discussion session.

Selected topics within those presented at the XVI International Pathogenic Neisseria Conference (XVI IPNC) will be discussed in this report, with special emphasis on *N. meningitidis* vaccine development. Advances in the *Neisseria gonorrhoeae* field will be discussed briefly at the beginning.

All data given in the present report have been published in detail in PubMed indexed articles or in the Abstract book of the XVI IPNC, available at http://www.ipnc2008.org.

Neisseria gonorrhoeae

Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection gonorrhea which is an acute inflammatory disease of the urogenital tract. Results presented in the XVI IPNC indicate that *N. gonorrhoeae* may establish infection in women by

1. Pollard AJ. Global epidemiology of meningococcal disease and vaccine efficacy. Pediatr Infect Dis J 2004;23:S274-9.

2. Perrett KP, Pollard AJ. Towards an improved serogroup B *Neisseria meningitidis* vaccine. Expert Opin Biol Ther 2005;5: 1611-25.

3. Finne J, Leinonen M, Makela PH. Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis. Lancet 1983;2:355-7.

4. Sierra GV, Campa HC, Varcacel NM, Garcia IL, Izquierdo PL, Sotolongo PF, *et al*. Vaccine against group B *Neisseria meningitidis*: protection trial and mass vaccination results in Cuba. NIPH Ann 1991;14:195-207.

5. Oster P, Lennon D, O'hallahan J, Mulholland K, Reid S, Martin D. MeNZB: a safe and highly immunogenic tailor-made vaccine against the New Zealand *Neisseria meningitidis* serogroup B disease epidemic strain. Vaccine 2005;23:2191-6.

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inhibiting the apoptotic response to infection, potentially avoiding host defenses and allowing time for intracellular replication. Several works reported that strains with decreased susceptibility to multiple antibiotics continue to be a growing problem. In the diagnosis field the need to consider genotypic methods was emphasized as an important supplement in the diagnosis of disseminated gonococcus infection, although culturing is still essential to obtain antibiotic susceptibility testing.

Many illnesses and infections in humans are exacerbated and/or caused by biofilms and is thought that nearly all chronic bacterial infections persist as biofilms. It has been found that gonococci form biofilm *in vitro* and during natural cervical infection [6]. Data presented in the XVI IPNC suggests that biofilm formation could minimize oxidative stress during natural cervical infection and allow *N. gonorrhoeae* to maintain a nitric oxide steady state that is non- or even anti-inflammatory and could lead to an asymptomatic infection in women, often leading to prolonged or chronic infections.

In the field of vaccine research, the ability of isolated oligosaccharide from the gonococcus was tested as a glycoconjugate vaccine, to elicit an IgG response specific for gonococci. The assayed vaccine was capable of eliciting a potent IgG response and a single dose of vaccine was sufficient to generate a maximal antibody response. It was also presented that immunization of mice with C3d fused to a peptide mimic of a conserved oligosaccharidic structure, increases the production of cross-reactive antibodies that were specific for the oligosaccharide epitope and were also bactericidal against a strain of *N. gonorrhoeae* that ordinarily resists killing by normal human serum.

New nomenclature for *Neisseria meningitidis*

The typing method currently in use for *N. meningitidis* is based on immunological approaches [7] that identified variants in the meningococcal capsule (serogroup), outer membrane proteins (serotype and serosubtype) and lipo-oligosaccharide (LOS) (immunotype). The availability of monoclonal antibody panels enhanced the utility of these methods, which proved valuable in a variety of applications. One of the main limitations of this serological scheme is the incomplete coverage and difficulties in production and provision of reagents, resulting in an increasing number of isolates not classifiable by these means [8].

In the 2008 Neisseria Conference, Dr. M. Maiden from the University of Oxford, UK, reminds us the proposal of a new typing scheme for *N. meningitidis*. It was proposed [9] to replace the serological approaches that determine phenotypes, currently in use, by nucleotide sequence-based approaches aimed at determining genotypes. Is it proposed that the new nomenclature adopted the form: serogroup: PorA type: FetA type: sequence type (clonal complex), thus (for example): B: P1.19,15: F5-1: ST-33 (cc32). This proposed classification conserved the capsule and PorA classifications, eliminates the PorB and LOS types from the nomenclature and incorporates the FetA type, the Sequence Type (ST) and the clonal complex (cc) classification.

Fet A was incorporated because it is always expressed *in vivo*, is present in most of clinical isolates, the FetA sequence diversity is confined to one variable region and currently FetA variable region peptides have been identified and categorized into families [10].

Multilocus sequence typing has been used to identify closely related strains with the potential to cause outbreaks. The system indexes the sequence variation of seven alleles of housekeeping genes that are under stabilizing selection and has enabled identification of groups of related genotypes that are referred to as cc, named after a predominant or central ST [11] . It has been shown that just seven cc, known as hyper invasive lineages, are responsible for the majority of meningococcal disease worldwide [12].

Epidemiology

Meningococcal disease can be endemic, causing sporadic isolated cases or small outbreaks within communities and institutions, or epidemic, spreading quickly through large populations. Importantly, *N. meningitidis* is currently the only bacterium that typically generates large epidemics of meningitis [13]. Each year, approximately 500 000 cases of meningococcal disease occur around the world, causing about 50 000 deaths. In developed countries, such as the USA and Europe, the rate of meningococcal disease ranges from 0.9 to 3.6 cases per 10^5 people. However, disease rates may be substantially higher in developing countries such as those within the African continent, with reported attack rates up to 100 per 10^5 person [1]. Meningococcal disease epidemiology is highly dynamic and unpredictable: the serogroup distribution varies from country to country and from region to region and changes over time. These changes can occur over relative short time periods [14].

The highest global incidence of meningococcal disease is caused by serogroup A, due to the large, cyclical epidemics in the so called "meningitis belt" of sub-Saharan Africa, where epidemic meningococcal meningitis continues to be a challenging public health threat, despite availability of control measures. In this region, the emergence of serogroups W135, X and Y has added complexity to the control of epidemic meningitis in Africa. The incidence of serogroup C disease has decreased overall after the introduction in several countries, such as United Kingdom, Canada, Netherlands and Italy, of massive immunization campaigns with conjugate serogroup C vaccines. In other such as Poland and Brazil, the incidence of serogroup C disease has increased.

In an increase in serogroup Y invasive meningococcal cases has been notified in Colombia since 2004, raising more than 30% of the cases. Additional increases have been also notice in Argentina and Costa Rica. Since the 1970s, serogroup B meningococcal disease has emerged and is now the most important serogroup in many countries worldwide. Serogroup B predominated from 1977-1998 in 20 different European countries and still causes 2/3 of the meningococcal disease burden in Europe. Endemic or hyperendemic serogroup B disease is reported from Australia and New Zealand, Canada and several South American countries, Africa outside the Meningitis Belt and the Near East. Major outbreaks caused by serogroup B are widespread and during outbreaks a specific serogroup B clone often dominates. Depending on the virulence potential of the dominating

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7. Frasch CE, Zollinger WD, Poolman JT. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. Rev Infect Dis 1985;7:504-10.

8. Feavers IM, Fox AJ, Gray S, Jones DM, Maiden MC. Antigenic diversity of meningococcal outer membrane protein PorA has implications for epidemiological analysis and vaccine design. Clin Diagn Lab Immunol 1996;3:444-50.

9. Jolley KA, Brehony C, Maiden MC. Molecular typing of meningococci: recommendations for target choice and nomenclature. FEMS Microbiol Rev 2007; 31:89-96.

10. Thompson EA, Feavers IM, Maiden MC. Antigenic diversity of meningococcal enterobactin receptor FetA, a vaccine component. Microbiology 2003;149: 1849-58.

11. Urwin R, Maiden MC. Multi-locus sequence typing: a tool for global epidemiology. Trends Microbiol 2003;11:479-87.

12. Caugant DA. Population genetics and molecular epidemiology of *Neisseria meningitidis*. APMIS 1998;106:505-25.

13. Girard MP, Preziosi MP, Aguado MT, Kieny MP. A review of vaccine research and development: meningococcal disease. Vaccine 2006;24:4692-700.

14. Stollenwerk N, Maiden MC, Jansen VA. Diversity in pathogenicity can cause outbreaks of meningococcal disease. Proc Natl Acad Sci USA 2004;101:10229-34.

clone, serogroup B outbreaks can differ substantially in severity of disease, mortality and sequelae of the affected population. In Argentine, serogroup B isolates are the most prevalent and represented 72% of all *N. meningitidis* isolates in 2006. Nevertheless, during the first five months of 2008, serogroup W135 increased dramatically, reaching the 27.7% of the isolated cases. In Brazil, five of the 7 hypervirulent lineages that have been circulating worldwide during the last 50 years were detected. The ST-32 was predominant in all geographic regions of the country. Studies on the analysis of the antigenic diversity among strains of *N. meningitidis* isolated before and after the immunization in Cuba with the OMV-based anti-meningococcal serogroup B vaccine VA-MENGOC-BC® were presented during the Conference in a study conducted by the Finlay Institute (Havana, Cuba).

Host-pathogen interaction

Several large exoproteins in various Gram-negative bacteria are predicted to be secreted via a two-partner secretion system. In the XVI IPNC was informed that *N. meningitidis* contains one of these systems, which contributes to the interaction of the bacterium with host epithelial cells.

A series of studies presented at the 2008 meeting demonstrated that the host laminin receptor mediates the contact between meningitis bacteria and the blood brain barrier; and that the major outer membrane porin PorA and the pilus secretin protein PilQ are the meningococcal ligands for the human laminin receptor.

Biofilm formation

Biofilm formation by *N. meningitidis* is a general trait of unencapsulated meningococcal variants which renders meningococci resistant to penicillin [15]. Data led to the assumption that meningococci persist asymptomatically in tonsillar tissue in a biofilm-like stage. It was found that extracellular DNA plays a key role for initial biofilm formation in some meningococcal strains.

IS1301

The identification of a serogroup C *N. meningitidis* strain resistant to the serum bactericidal activity (SBA) of vaccinees with polysaccharide: protein conjugate vaccines, prompted to investigate the basis of this resistance. Resistance resulted from the presence of an insertion sequence (IS), IS1301, in the intergenic region between the sia and ctr operons, necessary for capsule biosynthesis and export, respectively. The IS leads to an increase in the transcript levels of surrounding genes, which in turn leads to an increase the amount of capsule. The increased amount of capsule was associated with down-regulation of the alternative pathway of complement activation, providing a generic mechanism to protect the bacterium against bactericidal antibodies [16].

Antibiotics resistance

The increased resistance of *N. meningitidis* isolates to the antibiotics ciprofloxacin, rifampicin and penicillin was reported.

Diagnostics

In the field of diagnostics for *N. meningitidis* was presented a study analyzing the possibility of using current real-time PCR diagnostics, based on the Cu-Zn superoxide dismutase gene, sodC, found in *N. meningitidis* but not in any other *Neisseria* spp [17]. The current real-time PCR diagnostics for *N. meningitidis* target the capsule transport gene, *ctr*A [18]. However, over 16% of meningococcal carriage isolates lack the genes for capsule biosynthesis and transport. The sodC real-time PCR assay was a highly efficient, sensitive, and specific method for identification or detection of *N. meningitidis*, especially during carriage studies.

Vaccines

Correlates of protection

Goldschneider and colleagues were the first to demonstrate that an SBA titer of more than 4 was an appropriate both individual and population correlate of protection for meningococcal disease. They also demonstrated that an inverse relationship exists across different age groups between SBA against serogroup B meningococci and meningococcal disease [19].

Many factors may influence SBA titers: the choice of bacterial strain, growth conditions of the bacteria, the time of incubation with serum and the source of exogenous complement (human or rabbit sera). Baby rabbit complement gives substantially higher SBA titers. The explanation to this resides in that complement factor H (fH), that is a negative regulator of the alternative pathway, binds to *N. meningitidis* and increases resistance to serum bactericidal activity [20]. In a study presented at the XVI IPNC was found that binding is specific for human fH. When measured with rabbit complement, the decrease of bactericidal titers of tested human sera after addition of fH was more than 10-fold. Similar results were obtained with experiments *in vivo*. These data indicate that experiments that underscore the importance of binding of human fH for survival of *N. meningitidis in vitro* or *in vivo* do not reflect the actual functional activity of antibodies against *N. meningitidis* during human exposure, as for example, the results obtained of SBA assays, using non-human complement.

Due to the inherent variation in magnitudes of SBA titers between laboratories and the fact that most older children and adults have some pre-existing immunity to meningococcal disease, most studies of serogroup B vaccines have not relied on absolute SBA titers as a cut off, but use a greater than fourfold rise in SBA from pre- to postvaccination sera and this remains as the standard for comparison for serogroup B candidate vaccines, using exogenous complement from a person with no SBA antibodies and normal complement activity. However, due to the difficulties inherent to localize adequate volumes of human complement some researches agree that in the initial steps of antigens identification the rabbit complement could be used to screen within to a large quantity of antigens in the preclinical stage of research.

Serogroups A, C, Y, W-135

Polysaccharide-protein conjugate vaccines for the prevention of serogroup C meningococcal infection have proven efficacious: the incidence of the disease

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19. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129:1307-26.

20. Madico G, Welsch JA, Lewis LA, Mc Naughton A, Perlman DH, Costello CE, *et al*. The meningococcal vaccine candidate GNA1870 binds the complement regulatory protein factor H and enhances serum resistance. J Immunol 2006;177:501-10.

is lower in the countries that have introduced these vaccines into routine immunization schedules [21].

A quadrivalent meningococcal polysaccharide-diphtheria toxoid conjugate vaccine against serogroups A, C, W135 and Y, named Menactra® (Sanofi Pasteur, France) was licensed since 2005 in the USA and Canada to be used in 11- to 55-year-old people [22]. A similar vaccine, based on the conjugation of these four polysaccharides, but to CRM, MenACWY-CRM, was developed by Novartis. Results about tolerability and immunogenicity of this vaccine, investigated in five Phase II/III clinical trials, which included 4364 individuals from 2 months to 18-years-old of age, were presented at the XVI IPNC. MenACWY-CRM was well tolerated and immunogenicity was demonstrated in all age groups.

The cyclical epidemic of meningitis, mainly caused by *N. meningitidis* serogroup A, in the so called «meningitis belt» of sub-Saharan Africa, continues to be a problem. The Meningitis Vaccine Project (MVP), founded in 2001, is a partnership between WHO and the Program for Appropriate Technology in Health (PATH), that is funded by the Bill and Melinda Gates Foundation. The Director is Dr. F. Marc LaForce from France. The MVP objectives are the development, testing, introduction, and widespread use of an affordable Group A meningococcal conjugate vaccine in Africa. Dr. LaForce informed in the meeting that preclinical development finished in 2004 and the vaccine has been successfully tested in Phase I, II and II/III clinical trials in India and Africa (1-29 years). The vaccine has been shown to be safe and highly immunogenic. Introduction of the vaccine to public health scale in African countries is planned to begin in 2009 as a single dose in 1-29 year olds. The vaccine is priced at less than US$0.50 per dose, a price that guarantee a sustainable use.

Serogroup B

The formulation of a vaccine to prevent the serogroup B *N. meningitidis* disease represents a complex scientific challenge and remains an unmet medical need. The capsule is, by definition, the most conserved antigen within the serogroup, therefore a vaccine aimed to direct the immune response against the polysaccharide layer should protect against most isolates of this serogroup. Several strategies have been explored to design vaccines against serogroup B based on the CPS, a homopolymer of α2:8 linked sialic acid, but the development of such vaccines has been delayed due to the low immunogenicity of purified B polysaccharide. This has been attributed to the presence of some capsular epitopes in the bacteria shared with the carbohydrate occurring as part of the mammalian neuronal cell adhesion molecule, mainly found in the fetal brain and in small amounts in adult tissues. This has also raised the concern about the possibility that the antibodies elicited against this carbohydrate may exert autoimmune pathology [3]. However, until now there are no evidences for *in vivo* binding of anti-meningococcal polysaccharide B antibodies to human tissues or associated pathology [23, 24]. Nevertheless, most commercial companies have been unwilling to develop vaccines aimed to induce antibodies directed against the serogroup B CPS, but there are still some research groups working on this topic.

A strategy used to increase the immunogenicity of serogroup B polysaccharide was the replacing of the N-acetyl groups of the sialic acid residues in the bacterial polysaccharide with N-propionyl groups and the conjugation to tetanus toxoid (TT). The N-propionyl Group B polysaccharide-TT conjugate vaccine is immunogenic, elicits protective antibodies and a subset of antibodies do not cross-react with host antigens. The application of this strategy leads to the discovery of novel sialic acid epitopes, only present in the bacterial surface and not present in the mammalian carbohydrate, that could be the basis for a vaccine [25]. Moe and colleagues reported murine monoclonal antibodies, directed against the N-propionyl serogroup B polysaccharide-TT conjugate vaccine, which do not cross-react with human tissues, and are specifically directed to de-N-acetyl residues that are unintended side products produced during the polysaccharide propionylation [26]. The immunogenicity of protein-polysaccharide conjugate vaccines based on some of these derivatives was presented at the XVI IPNC. All neuraminic acid-containing polysaccharide conjugate vaccines were immunogenic and elicited antibodies of all classes and subclasses that were able to activate deposition of human complement on group B strains. Surprisingly, murine sera and monoclonal antibodies directed against the polysaccharide-derivatives were also reactive and mediated bactericidal activity against strains other than group B, having the potential to protect against strains from other capsular groups in addition to group B.

Another approach to meningococcal B vaccine development that has been exploited by the Centre for Genetic Engineering and Biotechnology of Havana, Cuba (CIGB) is the identification of peptides mimicking the immunogenic epitopes specific to the meningococcal B polysaccharide, that showed no cross-reactivity with human polysialic acid [27]. The CIGB presented a sequence analysis of several serogroup B CPS mimotopes, which have been able to induce antibodies with functional activity against *N. meningitidis* serogroup B.

Outer membrane vesicles-based vaccines

Neisseria meningitidis released blebs from the outer membrane, which contains the major outer membrane proteins, porins, phospholipids, LPS and lesser amounts of other proteins. This OMV can be prepared from cultures of meningococci using ultracentrifugation and detergent, as deoxycholate (DOC), extraction.

New-Zealand OMV-based vaccine

An overview of the impact of the MeNZB™ , an OMV-based vaccine developed and introduced in New Zealand to control an epidemic that started in 1991, was presented at the XVI IPNC by Dr. D. Martin from the Institute of Environmental Science and Research, Communicable Diseases of Porirua, New Zealand. The use of MeNZB™ to control New Zealand's epidemic of meningococcal disease was judged as successful. On the other hand, in a conference of the University of Auckland, Community Pedia-

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25. Jennings HJ, Roy R, Gamian A. Induction of meningococcal group B polysaccharide-specific IgG antibodies in mice by using an N-propionylated B polysaccharide-tetanus toxoid conjugate vaccine. J Immunol 1986;137:1708-13.

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27. Pon RA, Lussier M, Yang QL, Jennings HJ. N-Propionylated group B meningococcal polysaccharide mimics a unique bactericidal capsular epitope in group B *Neisseria meningitidis*. J Exp Med 1997; 185:1929-38.

trics of Auckland, New Zealand, was announced the decision of the New Zealand Ministry of Health to stop MeNZB™ vaccination mainly due to the reduction of meningococcal disease cases in the country. Since 2006/07 the vaccine was informally withdrawn, without resurgence of disease to date. This led to the recommendation that MeNZB™ be officially withdrawn since June 1st, 2008. In her conference, Dr. D. Martin manifested her concern in regard with this decision. She said that based on the longevity of the Norwegian epidemic for whose population a vaccine was not delivered, it is predicted that the New Zealand epidemic could continue for a further 5-15 years causing even greater mortality and morbidity and cited as example that Cuba controlled the epidemic with a strain-specific vaccine that is still include in the Cuban infant immunization program.

Netherlands vaccine

A recombinant nonavalent vaccine, consisting of three OMV, each containing three different PorA types, was developed by the Netherlands Vaccine Institute in an attempt to overcome the problem of antigenic variability of PorA protein among *N. meningitidis* strains. Results of a phase I trial to assess safety of NonaMen vaccine in 60 healthy adult volunteers was presented at the XVI IPNC and found that no major safety concerns arise during the study.

Vaccine against Neisseria lactamica

The Health Protection Agency Centre for Emergency Preparedness and Response, UK, presented during the conference the results of a Phase I safety and immunogenicity study of a meningococcal disease vaccine based on *N. lactamica* OMV, in adult volunteers. The vaccine was safe and increases in functional antibody responses were similar to those seen with a meningococcal OMV vaccine against heterologous meningococcal strains. As concluded by Dr. A. Gorringe, this vaccine showed some promise but is unlikely to be a successful «stand alone» meningococcal B vaccine.

New generation vaccines

The mining of information from genome sequence data and the application of the proteomic approaches has facilitated the discovery of novel vaccine antigens. Some of them have been selected by Novartis, Wyeth Vaccines Research (USA) and the Walter Reed Army Institute (WRAI, USA) as the basis of new generation vaccines that are in the final stages of preclinical testing or undergoing clinical trials.

Novartis vaccine

The complete genome of a virulent strain of *N. meningitidis* serogroup B (MC58) was explored at Novartis Laboratories to identify genes encoding potential bacterial surface proteins to be used in a broad-coverage vaccine, a process known as reverse vaccinology [28]. Following this strategy they have identified several novel serogroup B protein antigens, able to elicit bactericidal activity in mice, many of them highly conserved among meningococcal strains. The 5 most immunogenic antigens were selected for an experimental multivalent vaccine. Four of these antigens were combined into 2 fusion proteins so that the resulting protein vaccine contained 3 main recombinant proteins [29]: the factor H binding protein (fHBP) or GNA1870 (fused with GNA 2091), NadA and GNA2132 (fused with GNA1030). The final vaccine formulation, called Novartis MenB vaccine, also includes the New Zealand OMV due to their additional protective potential through *N. meningitidis* antigens as PorA and other, and due to the additional benefits due to the demonstrated adjuvant or immunostimulant effect of the OMV.

The fHBP or GNA1870 was first reported as identified by Novartis as a surface-exposed lipoprotein in the MC58 genome and three variants of fHBP were identified. Aminoacid identity within each variant is 91%-100%, while between variants ranges form 63%-85%. Antibodies directed against fHBP are bactericidal [30] and also facilitate bacterial killing and opsonophagocytosis by diverting fH away from the bacterial surface. The same protein was identified as LP2086 in the genome of the serogroup A strain Z2491 by Wyeth [31]. The vaccine candidate NadA protein [32] is a bacterial invasin that promotes adhesion to an invasion into epithelial cells . GNA2132 is present in most of *N. meningitidis* strains and also in *N. lactamica* and *N. gonorrhoeae* . It is able to confer protection against bacteremia in animal models and has been implicated in opsonophagocytic protection [33].

Results related with the characterization and immunological evaluation of vaccine antigens were presented in the XVI IPNC. It was demonstrated that recombinant proteins were immunogenic in neonatal mice when administered together as formulated in the vaccine. Antibody responses were significantly enhanced by adjuvants resulting in higher SBA titers and broader MenB strain coverage. These results are helpful to design optimal vaccination strategies against meningococcal disease in young people.

The results of Phase II clinical trial with the Novartis vaccine administered from 6 months of age, either alone or together with the New Zealand OMV vaccine were presented. Both vaccines were well tolerated. The best results were achieved with the combination of recombinant antigens with the New Zealand OMV vaccine; it was immunogenic when given as a 2 or 3 dose course from 6 months age against 3 strains expressing the vaccine antigens.

Wyeth vaccine

In an article published in 2004 was reported the identification in the Wyeth Vaccines Research laboratories of a neisserial outer membrane lipoprotein found in all *N. meningitidis* serogroup B strains tested. A gene encoding LP2086 was identified in their analysis of the *N. meningitidis* serogroup A Z2491 genomic sequence, and communicated that similar findings were reported with the genome-derived neisserial antigen GNA1870 described by researchers form Novartis. With the progress of the investigations it was revealed that both antigens are the same: the fHBP. LP2086 was classified in two distinct subfamilies based in amino acid sequence diversity derived from sequencing the LP2086 genes from 63 neisserial isolates [31].

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The imunogenicity of an experimental vaccine containing two recombinant lipidated fHBP representing the two sequence subfamilies (A and B) in cynomolgus macaques was studied. Bactericidal antibodies were elicited that killed strains within each meningococcal B LP2086 subfamily. Significantly higher SBA and IgG titers were observed with the inclusion of $AlPO_4$ adjuvant.

First-in-human studies performed in young adults (18 to 25 years) and adolescents (8 to 14 years) have shown that the vaccine elicits SBA responses against diverse serogroup B clinical isolates. A dose response was observed and the response rates (percentage of vaccines with at least 4-fold rise in SBA titer compared to pre-vaccination levels) against multiple isolates reached 100% for the highest dosage group. Most subjects reported only mild or moderate self-limiting adverse events.

The Walter Reed Army Institute (WRAI) vaccine strategy

Native OMV (NOMV) of *N. meningitidis* are highly immunogenic, but their high endotoxin content has prevented their use as a vaccine against group B meningococci. Furthermore, first generation of OMV vaccines prepared with DOC provided relatively narrow mostly subtype-specific immunogenicity.

With the objective to develope a vaccine based on NOMV that will be safe and provide broad based protection against meningococcal group B strains, researches from WRAI selected three antigenically diverse group B strains to introduce genetic modifications to improve safety and to increase and stabilize the expression of desirable antigens. The modifications consisted in:ΔlpxL1 (to reduce LOS associated toxicity); ΔsynX (to achieve capsule-negative, non-sialylated LOS phenotype); ΔlgtA (to eliminate phase-variable expression of lacto-N-neotetraose, and to stabilize expression of the short-chain LOS immunotypes). Each strain was also modified to express a second, heterologous PorA. Expression of minor conserved outer membrane proteins (NadA and variants 1 and 2 of fHbp) was increased in each of the three strains to achieve broader antigenic cross-reactivity. All genetic modifications demonstrated stability during at least 6 observed passages.

The modified NOMV vaccine strains were found to produce NOMV that were non-pyrogenic and at a consistent level with safety in humans. Also, tests involving the release of pro-inflammatory cytokines from whole human blood suggest that the vaccine will be safe for parenteral administration in humans. The combined NOMV vaccine was used to immunize mice and rabbits and induced a four-fold or greater increase in bactericidal antibodies against homologous and most of heterologous bactericidal test strains. A clinical lot of NOMV vaccine was prepared from one of the three vaccine strains, preclinical testing was done, and a phase 1 study is in progress as a probe of principle to demonstrate safety and immunogenicity in humans.

New antigen discovery and/or characterization

The identification and characterization of new potential universal vaccine antigens or the characterization of already-known antigens was also presented at the meeting. The studies about some of them are presented in the following paragraphs.

The majority of serogroup B and C disease in the past five decades was caused by a small number of hyper invasive lineages that remains stably associated with limited combinations of Opacity (Opa) proteins. The role of Opa in facilitating entry into host cells has been clearly demonstrated [34]. In the XVI IPNC fourteen *opa* genes were cloned, expressed in *Escherichia coli*, refolded, purified and used to immunize mice. Immunization with the panel of recombinant Opa proteins induced bactericidal antibodies against the majority of meningococci from a collection representing the hyper invasive lineages.

The potential of Haemoglobin receptor (HmbR), which enables *N. meningitidis* to use hemoglobin as a source of iron [35], in future vaccine research was highlighted. A statistically significant association between disease and the presence of the gene among the hyper-virulent cc was found.

The analysis of the TonB-dependent receptors [36] in the pathogenic Neisseriae, revealed that one of them, NMB0964, is regulated by Zn. This was the first report of a TonB-dependent receptor that is regulated by zinc. In the human body there is a zinc-limiting environment; NMB0964 will therefore be expressed in the host. This, together with its high conservation and its ability of inducing bactericidal antibodies, makes this protein a good vaccine candidate.

In the outer membrane of *N. meningitidis* was described the presence of the glycolytic enzyme fructose bisphosphate aldolase, in a study that constitutes the first report of the presence of this enzyme in the outer membrane of a Gram negative organism. The meaning of the presence of this enzyme in the meningococcal outer membrane and its possible importance in the immune response against *N. meningitidis* is now under investigation.

The identification of novel protein vaccine candidates is one of the strategies followed by the CIGB to formulate a universal meningococcal serogroup B vaccine. To accomplish this they have combined proteomic technology [37] with *in silico* prediction of candidates and the application of expression library immunization [38]. The lipoprotein NMB0928 [39], is a minor surface-exposed cross-protective anti-meningococcal serogroup B candidate vaccine. In the XVI IPNC was presented a strategy that increased the cross-immunogenicity of OMV vaccines by the incorporation of this recombinant antigen in the OMVs.

The meningococcal antigens NMA0939 and NMB0938 were identified at the CIGB as potential vaccine candidates. It was found that their genes were present in the 100% of the strains assessed and the overall identity of the deduced polypeptides ranged from 95 to 100%. Both antigens induced a functional response in mice, characterized by the presence of bactericidal antibodies also able to protect against meningococcal bacteremia in the infant rat model.

The vaccine potential of the surface-exposed protein NMB0088 (or OmpP1) of *N. meningitidis* has been also explored at the CIGB. A proposed structural model for the antigen suggests that NMB0088 is a beta-barrel protein with 7 exposed loops and 14 membrane

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37. Uli L, Castellanos-Serra L, Betancourt L, Dominguez F, Barbera R, Sotolongo F, *et al*. Outer membrane vesicles of the VA-MENGOC-BC vaccine against serogroup B of *Neisseria meningitidis*: Analysis of protein components by two-dimensional gel electrophoresis and mass spectrometry. Proteomics 2006;6:3389-99.

38. Yero CD, Pajon FR, Caballero ME, Cobas AK, Lopez HY, Farinas MM, *et al*. Immunization of mice with *Neisseria meningitidis* serogroup B genomic expression libraries elicits functional antibodies and reduces the level of bacteremia in an infant rat infection model. Vaccine 2005; 23:932-9.

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spanning segments. Four variants of NMB0088 were identified and variability was found to be confined to three specific segments, designated VR1, VR2 and VR3, putatively located within or near the predicted extracellular looping domains. Purified recombinant NMB0088 from one serogroup B strain was used to immunize mice and 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, thus vaccine preparations based on this antigen should be carefully designed in order to provide broad coverage against *N. meningitidis.*

These immunological studies with the CIGB antigens were conducted in adult mice, but they have also explored the early life immunity of the individual protein antigens using neonatal mice, an animal model that resembles the immature immune state of human infants [40]. All antigens were immunogenic and some of them elicited a broader cross-reactive response at this age.

Proteomics

An interesting immunoproteomic analysis of the development of immunity in response to colonization by *N. meningitidis* with the aim of identifying potential vaccine targets was presented. Proteins from the meningococcal outer membrane of homologous and heterologous serogroup B strains were probed with panels of sera obtained prior to and post colonization detection. The combination of 2-D SDS-PAGE with Western blotting allowed the identification of antigens capable of inducing cross-reacting antibodies involved in the development of natural immunity to meningococci.

The proteomes of *N. meningitidis* growth in biofilms and planktonic were compared and concluded that protein expression changes during meningococcal biofilm formation.

Genomics

A genome of *N. lactamica* was fully compared with the genomes of three *N. meningitidis* isolates (Z2491, MC58, FAM18) and the *N. gonorrhoeae* isolate FA1090. The high degree of similarities among these genomes is remarkable, considering that *N. lactamica* is a harmless commensal while *N. meningitidis* and *N. gonorrhoeae* have the potential to cause serious disease.

The whole-genome sequence of three meningococcal carriage isolates were obtained and compared to the genome sequences from three disease isolates already available in public databases as well as to the genome sequences from *N. gonorrhoeae* and *N. lactamica*, respectively [41]. The results suggested that *N. meningitidis* might have emerged as an unencapsulated human commensal from a common ancestor with *N. gonorrhoea* and *N. lactamica* and acquired the genes responsible for capsule synthesis within evolutionary recent times.

The CIGB presented their results of *in silico* identification of new potential vaccine antigens within the *N. meningitidis* genome sequence. A number of novel proteins, with potential outer membrane localization were identified and 7 were experimentally tested. Some of them elicited antibodies with bactericidal activity after immunization. This study demonstrated that utilization of genome sequences by applying bioinformatics is still possible to expedite the vaccine discovery process in *N. meningitidis.*

The genetic elements differing between disease- and carriage associated meningococcal isolates were identified by subtractive hybridization comparisons between selected disease-associated and carriage-associated strains. Several genes were identified as being present in the disease strain and absent in the carrier strain.

Conclusions

The XVI IPNC was a meeting that enabled fruitful discussion and provided participants a significant amount of novel information. Many subjects were covered, with special emphasis on vaccine development. Particularly interesting were the advances to fight the disease against all serogroup B strains. The combination of new approaches with conventional vaccine strategies, as discussed in the meeting, has brought new potential vaccine candidates to preclinical and clinical trials. Also, progress to solve the meningococcal group A meningitis in sub-Saharan Africa are hopeful.

The contributions of the XVI IPNC conference are hoped to enhance basic scientific knowledge and advance vaccine developments.

Acknowledgments

We thank to Karla Rutten (Congress Care, Netherlands) for giving us the data of participants and the countries they represented.

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