Conjugate and Polysaccharide Vaccines
**Meningococcal polysaccharide-protein conjugate vaccines**

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Meningococcal polysaccharide vaccines are available for prevention of invasive diseases caused by *Neisseria meningitidis*, serogroups A, C, Y and W135. Protection correlates with the ability of vaccination to induce serum complement-mediated bactericidal antibody(1) In general, the antibody responses to these unconjugated polysaccharides are age-dependent: these vaccines are highly effective in adults (1), but they elicit negligible or incomplete and short-lived protection in infants and preschool children (1,2). Polysaccharide vaccines for prevention of disease caused by serogroup B meningococcal strains are not available. The group B polysaccharide is a poor immunogen at all ages, possibly because of immunologic tolerance induced in the host by the presence of cross-reactive polysialyated glycoproteins in fetal and adult tissues (4).

Most polysaccharides appear to elicit antibody responses largely without the need for T-cell help (i.e., they are thymic-independent, or TI antigens). Conjugation of a polysaccharide to a protein carrier profoundly alters the immunologic properties of the polysaccharide, converting it from a TI to a thymic-dependent (TD) antigen. In the resulting TD conjugate, the immunogenicity of the polysaccharide is greatly enhanced, giving rise to IgG anticapsular antibodies and memory B cells.

**Experience with *Haemophilus* type b conjugate vaccines.** Much has been learned about human immunity to polysaccharide-protein conjugate vaccines from studies of *Haemophilus influenzae* type b (Hib) vaccines. Compared to unconjugated Hib polysaccharide, the immunogenicity of the conjugated Hib polysaccharide in infants is greatly enhanced, and repeated injections elicit IgG booster responses, whereas such responses are not observed after repeated doses of unconjugated Hib polysaccharide (1). Serum antibody to the type b capsular confers protection against invasive Hib disease. In addition, Hib conjugate vaccination primes for long-term immunologic memory to the Hib polysaccharide, a property not elicited by vaccination with unconjugated Hib polysaccharide or even after recovery from Hib disease (1). The ability to develop memory B cells, which leads to rapid IgG anticapsular antibody responses upon encountering Hib organisms, may be an important alternative mechanism of protection against developing disease in vaccinated individuals who either have shown subprotective antibody responses to Hib conjugate vaccination (1), or whose serum antibody concentrations have declined to below the protective level (1).

Hib conjugate vaccines are effective in two other important ways: first, the anticapsular antibody elicited by repeated injections of Hib conjugate vaccines undergoes affinity maturation (author’s unpublished data). The resulting higher avidity antibodies are more efficient at activating complement-mediated bacteriolyis or opsonization of Hib than lower avidity antibodies (1,2). Second, immunization with Hib conjugate vaccines not only protects the individual from developing invasive Hib disease by inducing protective immunity, but also
lowers the rate of nasopharyngeal colonization and transmission of Hib in the population (1,2). By this mechanism, Hib conjugate vaccination can have a better effect on decreasing the incidence of Hib disease in the population than would be predicted based on vaccine coverage (1).

**Experience with meningococcal polysaccharide-protein conjugate vaccines.** Meningococcal oligosaccharide- and polysaccharide-protein conjugate vaccines have been prepared for prevention of diseases caused by serogroups A, B, and C organisms (1,2,3,4,5). To date, data from humans are limited to serogroup A and C meningococcal oligosaccharide-protein conjugate vaccines. A first-generation prototype vaccine was prepared at Chiron Biocine using meningococcal A and C oligosaccharides that are independently coupled to CRM<sub>197</sub> carrier protein (a cross-reactive nontoxic mutant diphtheria toxin) (17). The conjugation method is based on selective end-reducing group activation of oligosaccharides and subsequent coupling to the protein through a six-carbon “spacer” molecule, adipic acid. In adults, the immunogenicity of the first-generation meningococcal A and C conjugate vaccine appeared to be similar to that of a control unconjugated meningococcal polysaccharide vaccine (1). A “second-generation” meningococcal C vaccine was prepared using similar chemistry except that very small oligomers (degree of polymerization [Dp] less than six monomers in length) were removed prior to conjugation (1). In phase I and II clinical trials in humans, both vaccines have been shown to be very safe in infants, toddlers and adults (1,2,3,4,17,18,19). Expanded clinical trials with the second-generation meningococcal C conjugate vaccine are currently in progress.

**Immunogenicity. Toddlers.** In a study of US toddlers 18 to 23 months of age, conducted at the University of California, Los Angeles (UCLA), two doses of the Chiron Biocine meningococcal A and C conjugate vaccine given two months apart elicited 50- to >100-fold higher bactericidal antibody responses to both group A and group C strains, compared to those observed in control toddlers vaccinated with two doses of unconjugated meningococcal polysaccharide vaccine (Table 1) (21). Interestingly, the relative differences in immunogenicity between the conjugate and unconjugated vaccines in this study were much less striking when the antibody responses of the toddlers were measured by an ELISA, performed using a consensus protocol developed at the Centers For Disease Control (i.e., relative differences in antibody responses of 2-fold, instead of 50- to >100-fold, as determined by the bactericidal assay). These results suggest that the antcapsular antibody elicited by this conjugated meningococcal oligosaccharide vaccine is qualitatively different from that elicited by unconjugated meningococcal polysaccharide vaccine and, on a µg/ml basis, the conjugate-induced antibodies have a higher specific functional activity. Further, the ELISA is insensitive to these qualitative antibody differences, which may be important in protection against developing meningococcal disease.

**Table 1. Serum bactericidal antibody responses of US toddlers vaccinated with a meningococcal A and C oligosaccharide-CRM conjugate vaccine**

<table>
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<tr>
<th>Geometric Mean Bactericidal Titer (Reciprocal)</th>
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Toddlers were given two doses of either meningococcal A and C oligosaccharide-CRM$_{197}$ conjugate vaccine, prepared at Chiron Biocine, or meningococcal polysaccharide vaccine (Menomune, Connaught Laboratories, Inc.). The injections were separated by two months. Complement-mediated bactericidal activity was measured in serum obtained immediately before dose one (Pre-) and one month after dose two (Post-2). Adapted from Lieberman J, et al. JAMA 1996;275:1499–1503. (21)

The “second-generation” Chiron Biocine meningococcal C polysaccharide-protein conjugate vaccine, in which small saccharide oligomers are removed prior to conjugation, was recently evaluated in toddlers 12 to 23 months of age in a multicenter Canadian study (19). This vaccine also elicited much higher serum bactericidal antibody responses after one or two injections than those observed in sera of control toddlers vaccinated with unconjugated polysaccharide vaccine.

**Infants.** The safety and immunogenicity of Chiron Biocine meningococcal conjugate vaccines also have been investigated in infants less than six months of age. The first study was performed with the combined A and C conjugate vaccine in Gambian infants immunized at 2, 3 and 4 months of age, or 2 and 6 months, or 6 months of age (20). The antcapsular antibody responses of the infants to the A component of the conjugate vaccine were of similar magnitude to those of a control group receiving an unconjugated meningococcal A and C polysaccharide vaccine. In contrast, the conjugate vaccine elicited two- to four-fold higher antcapsular antibody responses to meningococcal C polysaccharide than those observed in infants given the unconjugated polysaccharide vaccine. However, the elevated anti-C antibody concentrations in the conjugate group began to decline within three months after vaccination.

In the Gambian study, only ELISA antibody responses were reported. Based on the experience in the UCLA toddler study described above (21), the ELISA results may have underestimated the relative effectiveness of the conjugate vaccine as compared to unconjugated polysaccharide, had the responses been assessed by serum bactericidal antibody. In a study in the UK, 58 infants were vaccinated with the combined Chiron Biocine meningococcal A and C conjugate vaccine at 2, 3 and 4 months of age (23). To date, antibody responses to the group A vaccine have not been evaluated. However, the infants showed excellent anti-meningococcal C bactericidal responses after the first and second injections (geometric mean bactericidal titer of <1:10 prior to...
vaccination increasing to >1:100 after one injection, and >1:1000 after two injections). There was no further increase in titer after the third injection.

There are two other reports of the use of meningococcal C conjugate vaccines in infants. In one study, the “second-generation” Chiron Biocine meningococcal C polysaccharide-protein conjugate vaccine was given to UK infants at 2, 3 and 4 months of age (22). Preliminary results were limited to assays of sera obtained prior to vaccination and one month after the third injection. The infants showed 25-fold increases in anticapsular antibody concentrations, as measured by ELISA, and >50-fold increases in bactericidal titers. In the other study, a meningococcal serogroup C oligosaccharide-CRM 197 conjugate vaccine was prepared by Lederle Praxis Biologics and administered to US infants at 2, 4 and 6 months of age (16). This conjugate vaccine appeared to be well tolerated and elicited significant increases in serum anticapsular antibody concentration after two or three injections, as assessed by ELISA.

**Induction of immunologic memory.** In the study of Gambian infants given the Chiron Biocine first-generation combined meningococcal A and C vaccine, serum antibody concentrations to both polysaccharides had begun to decline within three months after conjugate vaccination (20). An important question, therefore, is whether or not the conjugate vaccination induced memory B cells that might allow the infants to respond rapidly with an increase in serum anticapsular antibody concentration upon encountering group A or C meningococci. To examine this question, participants in this study were re-vaccinated at 18 to 24 months of age with an unconjugated meningococcal polysaccharide vaccine (1). Prior to the booster injection, the serum antibody concentrations to the A or C polysaccharides in the groups previously given the conjugate vaccine were not significantly different from those of toddlers of similar age who had not been previously vaccinated. However, as shown in Table 2, the toddlers primed with meningococcal conjugate vaccine at 2 and 6 months of age developed much higher anti-meningococcal C bactericidal titers after the polysaccharide booster vaccination than did toddlers of similar age immunized for the first time with unconjugated meningococcal polysaccharide vaccine. In contrast, the Gambian toddlers who had been primed with meningococcal unconjugated polysaccharide vaccine at 3 and 5 months of age showed significantly lower anti-C bactericidal responses to the booster injection than the control toddlers vaccinated for the first time (Table 2). This result confirms previous data suggesting that immunization at an early age with unconjugated meningococcal group C polysaccharide vaccine induces immunologic tolerance and impairs the ability to respond to a subsequent immunization with unconjugated meningococcal polysaccharide vaccine (1). The clinical importance of this finding is unknown. However, immunologic tolerance to meningococcal C polysaccharide could enhance susceptibility to developing invasive meningococcal disease by impairing the ability of the child to develop a protective serum anticapsular antibody response upon encountering the organism.

Although the Gambian toddlers who had been given conjugate vaccine as infants were primed for memory antibody responses to meningococcal C polysaccharide, similar priming was not observed to the group A polysaccharide: that is, the magnitude of the anti-A antibody response to the booster injection of unconjugated polysaccharide vaccine in the conjugate-primed group was not significantly higher than that observed in Gambian toddlers vaccinated with unconjugated meningococcal polysaccharide for the first time (Table 2). Further, in contrast to meningococcal C, evidence of immunologic tolerance to meningococcal A polysaccharide was
not observed in the toddlers previously given the unconjugated polysaccharide vaccine at 3 and 5 months of age (Table 2). Indeed, the toddlers previously vaccinated with unconjugated polysaccharide vaccine appear to have shown secondary antibody responses to the serogroup A polysaccharide. These data confirm the results of many previous studies indicating that group A and group C meningococcal unconjugated polysaccharide vaccines have very different immunologic properties (summarized in reference 4, Frasch, 1995): specifically, meningococcal A polysaccharide vaccines appear to be both immunogenic and protective in early infancy (2), and may even prime for secondary antibody responses to a subsequent injection (25). Meningococcal C polysaccharide vaccine shows none of these properties. The immunologic basis for these differences remains unknown.

Recently, toddlers who participated in the UCLA study of the Chiron Biocine meningococcal A and C conjugate vaccine were also given a booster injection of unconjugated meningococcal polysaccharide vaccine approximately one year later (1). The group that had been primed with the conjugate vaccine showed evidence of induction of memory B cells to both the serogroup A and C polysaccharides, as evidenced by very high serum bactericidal booster antibody responses. These results are in contrast to those of the Gambian infants given this conjugate vaccine in whom evidence of induction of memory B cells was limited to the serogroup C polysaccharide, and not the serogroup A polysaccharide (see above) (24). Further studies of immunologic priming induced by meningococcal A conjugate vaccines at different ages and in different populations are needed to clarify this discrepancy.
Table 2. Effect of priming with meningococcal A and C oligosaccharide-CRM conjugate vaccine on serum bactericidal antibody responses of Gambian toddlers boosted with unconjugated meningococcal polysaccharide vaccine*

<table>
<thead>
<tr>
<th>Geometric Mean Bactericidal Titer (Reciprocal) 10–14 Days Post-Booster</th>
<th>Geometric Mean Bactericidal Titer (Reciprocal) 10–14 Days Post-Booster</th>
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<tbody>
<tr>
<td>Meningococcal Bactericidal Antibody</td>
<td>Priming Vaccine</td>
</tr>
<tr>
<td>None (N = 34)</td>
<td>Polysaccharide Vaccine (N = 17)</td>
</tr>
<tr>
<td>Anti-A</td>
<td>338</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-C</td>
<td>239</td>
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*Toddlers in the Gambia were boosted with a dose of unconjugated meningococcal polysaccharide vaccine (Menpovax A plus C, Biocine, Siena, Italy) at a mean age of 19.7 months. The subjects had either been previously vaccinated at 3 and 6 months of age with unconjugated meningococcal polysaccharide, or at 2 and 6 months of age with meningococcal A and C oligosaccharide-CRM conjugate vaccine (Twumasi, et al.) (20). A group of control unprimed toddlers from the same study area were vaccinated for the first time. Data shown are bactericidal titers measured in sera obtained 10 to 14 days after the booster vaccination (adapted from Leach A, et al., J Infect Dis 1996, in press) (24).

Meningococcal B polysaccharide-protein conjugate vaccines. Investigational polysaccharide-protein conjugate vaccines also have been prepared for prevention of disease caused by serogroup B organisms (1,2,3,15). In general these conjugates are much less immunogenic than those prepared with serogroup A or C polysaccharides. However, one promising immunogenic meningococcal B vaccine candidate is a conjugate in which the polysaccharide component has been modified by substitution of N-propionyl groups for N-acetyl groups (NPr-meningococcal B polysaccharide) (28). To date, there are no published data from trials in humans with this type of conjugate. However, it will be a difficult task to prove that such vaccines are safe in humans because, in mice, data from our laboratory suggest that NPr-meningococcal B polysaccharide-protein conjugate vaccines induce anti-NPr-meningococcal B polysaccharide antibodies that cross-react with native NAc-meningococcal B polysaccharide and also appear to have autoantibody activity (Bartoloni A, et al., unpublished data). Whether or not the use of alternative substitutions, such as N-butanoyl, will truly permit an antibody response that is functional, entirely pathogen-specific, and not cross-reactive with host antigens, remains to be ascertained (1).
Conclusions and the future. Meningococcal A and C conjugate vaccines hold enormous promise for providing solid long-term protection to infant age groups that are currently poorly protected by licensed unconjugated meningococcal polysaccharide vaccines. Further, “third-generation” conjugate vaccines are under development in which the acquisition of immunity is accelerated and immunogenicity is enhanced by administration of the conjugate vaccine with novel adjuvants (1). The use of adjuvants suitable for humans holds promise for decreasing the number of doses of conjugate vaccine required for induction of immunity in infants. This approach also may be useful for enhancing or maintaining immunogenicity of future multicomponent conjugate vaccines. For prevention of group B meningococcal disease, adjuvanted polysaccharide-protein conjugate vaccines also may prove to be immunogenic and protective. However, alternative approaches may be needed to avoid the safety concerns of inducing anticapsular antibodies with autoantibody reactivity.

Acknowledgement Supported, in part, by a grant (V23/181/169) from the World Health Organization Global Programme for Vaccines.

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**Protective epitope of N-propionylated group B meningococcal polysaccharide.**

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*Neisseria meningitidis* is a human pathogen of worldwide significance. Although group B *N. meningitidis* is the most pathogenic serogroup its capsular polysaccharide is precluded from the current polysaccharide vaccine due to its poor immunogenicity (1). Furthermore this problem, which is attributed to structural mimicry between the group B meningococcal polysaccharide (GBMP) and human tissue antigens (2), cannot be satisfactorily overcome by coupling the GBMP to protein carriers (3). Currently there is no vaccine against group B meningococcal meningitis and most efforts to develop an effective vaccine have focused on sub-capsular components such as outer membrane proteins and lipopolysaccharides. Problems have been encountered in the development of these vaccines not the least of which is the intrinsic antigenic diversity exhibited by the components (4). Because the GBMP is a conserved antigenic structure on group B meningococci, a polysaccharide-based vaccine would be the vaccine of choice, provided one could overcome its poor immunogenicity.

One simple way to achieve this goal is to use a synthetic vaccine composed of the N-propionylated (NPr) form of the GBMP, which when conjugated to tetanus toxoid (TT), induces in mice high titer antibodies that are bactericidal for all group B meningococci (5). The NPr-GBMP-TT produced two distinct populations of antibodies, one of which (minor population) cross-reacted with the GBMP. Of significance to the development of a vaccine was that the major population of antibodies did not cross-react with the GBMP and yet contained all the bactericidal activity towards group B meningococci (6). In addition the induction of GBMP cross-reactive antibodies could also be reduced by adjuvant manipulation. Thus the NPr-GBMP must mimic a unique epitope on group B meningococci (6).

In order to further define this epitope a series of mAb's of the IgG isotype were produced in BalbC mice using an (NeuPr)~35-TT conjugate vaccine. Most of the mAb's which were only minimally cross-reactive with the GBMP, recognized an extended helical form of the NPr GBMP. However, unlike GBMP-specific antibodies, which only recognize extended helical epitopes on the GBMP (2), a few were able to recognize short (random coil) segments of the NPr GBMP. Because of the paucity of clones specific for these short epitopes, additional mAb's with this specificity were generated using an (NeuPr)4-TT conjugate. Two important conclusions can be drawn from the properties of the above mAb's. The first is that while antibodies of the IgG1 isotype are not bactericidal, they confer good passive protection in mice challenged with live group B meningococci. The second is that regardless of isotype only mAb's specific for the extended helical form of the NPr GBMP are protective as defined by either passive protection experiments or bactericidal activity. Therefore one can draw the intriguing conclusion that whereas the serologically distinct extended helical epitopes of both the GBMP and the NPr GBMP co-exist in the capsular layer of group B meningococci and *E. coli* K1, only the former are present in purified α2-8-polysialic acid.
The presence of extended NPr GBMP-specific epitopes in the capsular layer of the above organisms was substantiated by electron microscopy, using a mAb with this specificity as the binding antibody. In addition using this technique, it was demonstrated that a mAb specific for short NPr GBMP epitopes did not bind to either organism, which is consistent with the lack of protection provided by mAb's with this specificity.

References

Preclinical evaluation of a combination vaccine against groups A, B, and C meningococci in both mice and nonhuman primates

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*Neisseria meningitidis* is the major cause of bacterial meningitis worldwide. Meningococci of serogroups A, B, and C are responsible for approximately 90% of all the reported cases (1, 2). A combination polysaccharide-protein conjugate of each serogroup (A, B, and C) is being developed as a vaccine candidate against meningococcal infections.

For group B, the polysaccharide was chemically modified at the C-5 position of the sialic acid residue wherein the N-acetyl groups are replaced with N-propionyl groups to alter the immune tolerance and provide greater immunogenicity (3, 4). The chemically modified B polysaccharide, as well as the native A and C polysaccharides, were coupled to the carrier protein, a recombinant class 3 porin (rPorB) of group B meningococci (5), by reductive animation (3). The rPorB was chosen as the carrier protein due to its ability to significantly increase the bactericidal activity towards the group B polysaccharide when conjugated (6, manuscript submitted for publication).

The groups A, B, and C conjugates were evaluated individually and in combination in both mice and nonhuman primates (African green monkeys). Immune responses were assessed in terms of polysaccharide-specific IgG (by ELISA) and antibody-dependent complement-mediated bactericidal activity.

In mice, the combination vaccine is highly immunogenic, eliciting high levels of polysaccharide-specific IgG and bactericidal activity against all three components. Booster effects were also clearly demonstrated after subsequent injections for all components indicating that T-dependency was achieved. No significant interference in immunological responses was observed for the trivalent vaccine formulation when compared with the monovalent vaccine controls.

The responses in nonhuman primates for individual and combination vaccines are being evaluated, but initial results have shown significant bactericidal activity against all 3 serogroups after only one injection of the trivalent formulation.

References


Conjugate and Polysaccharide Vaccines

Peptide mimic-induced primary human antibody response to the capsular polysaccharide of *Neisseria meningitidis* serogroup C

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Recent trials with a conjugated meningococcal vaccine preparation have failed to show enhanced immunogenicity over the conventional capsular polysaccharide vaccine (1). An alternate approach to convert the T-independent meningococcal vaccine into a T-dependent vaccine is through the use of an anti-idiotypic mimic of the native antigen. We have developed an anti-idiotypic-based peptide mimic of the capsular polysaccharide of *Neisseria meningitidis* serogroup C (MCPS). Immunization with this peptide complexed to proteosomes results in a protective anti-MCPS antibody response in Balb/c mice (2). Study of the human immune system has been hampered by the lack of experimental models to generate a primary immune response to T-independent or T-dependent antigens. Mosier et al. (3) demonstrated mutant severe combined immunodeficient (SCID) mice could be engrafted with functional human peripheral blood lymphocytes (hu-PBL). A major limitation of the hu-PBL-SCID model has been the failure to demonstrate a primary human immune response (4-8). We hypothesized that the lack of consistent human primary immune response may be attributed to the lack of human cytokines resulting in impaired differentiation and maturation of human lymphoid cells in reconstituted SCID mice. We have developed a reliable system of inducing a human primary antibody response in the reconstituted hu-PBL SCID mouse model (9). This study was undertaken to define the optimal dose and configuration of MCPS peptide mimics.

Three healthy volunteers were leukopheresed. Fifty five SCID mice/volunteer were reconstituted with $10^8$ human lymphocytes and immunized with 10 µg MCPS; 10, 25, 50, or 100 µg of the P3 peptide (CARIYYRYDGFAY) complexed to proteosomes; 25 or 50 µg of 3xP3 peptide (IYYRYDIYYRYDIYYRYD) complexed to proteosomes; 25 or 50 µg of 3xYPY peptide (IYYPYDIYYPYDIYYPYD) complexed to proteosomes. The human anti-MCPS response was measured by ELISA. Functional activity was determined by bactericidal assay.

The results of these studies showed that immunization with 50µg of P3 peptide complex or 25 µg of 3xP3 peptide complex resulted in the highest human anti-MCPS antibody titer (21.5 and 21.4 µg/ml respectively). Immunization with the 3xYPY peptide resulted in 10-15 µg/ml human anti-MCPS antibody. Immunization with MCPS (one dose) results in 0.26 µg/ml anti-MCPS at 4 weeks. All antisera with an anti-MCPS titer exceeding 1 µg/ml proved to be functional in bactericidal assay. These data indicate that an anti-Id based peptide mimic of MCPS induces a protective, T-dependent antibody response in humans.

References

Murine monoclonal antibodies to an N-propionylated meningococcal B polysaccharide exhibit heterogeneity with respect to cross-reactivity with N-acetylated meningococcal B polysaccharide and autoreactivity to host polysialyated glycoproteins

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The poor immunogenicity of the Neisseria meningitidis group B (MenB) polysaccharide capsule (PS), a homopolymer of α 2-8 sialic acid, represents a major challenge in developing an effective polysaccharide-based vaccine to prevent MenB disease. The poor immunogenicity of MenB PS has been attributed to immunologic tolerance induced from exposure to host polysialyated glycoproteins (e.g., neural cell adhesion molecules, termed N-CAMs). Substitution of N-propionyl (NPr) for N-acetyl (NAc) groups on the MenB PS, and conjugation of the resulting NPr MenB PS to a protein carrier, has been reported to result in a conjugate vaccine that is immunogenic in experimental animals and capable of eliciting protective antibodies that activate complement-mediated bactericidal activity (1). However, little is known about the cross-reactivity of anti-NPr MenB PS antibodies with NAc MenB PS, or autoreactivity of these antibodies. To address these questions, we raised a panel of 28 murine anti-NPr MenB PS antibodies. After partial purification of the Mabs from tissue culture supernatants by ammonium sulfate fractionation and exhaustive dialysis, the Mabs were characterized for isotype, cross-reactivity with NAc MenB PS by ELISA, and autoreactivity with a neuroblastoma cell line (CHP-134), which has been reported to express long chain α 2-8 linked polysialic acid (2). Of the 28 Mabs, one was IgM and the remaining 27 were IgG (three IgG1, three IgG2a, thirteen IgG2b and eight IgG3). Fourteen of the 28 antibodies (50%) cross-reacted with NAc MenB PS as demonstrated by direct binding to NAc MenB PS in a solid phase ELISA format. The specificity of this cross-reactivity was confirmed by inhibition of binding with soluble NAc MenB PS. The remaining 14 Mabs showed no cross-reactivity with NAc MenB PS when tested by ELISA at antibody concentrations up to 25 μg/ml. In preliminary studies, complement-mediated bactericidal activity was detected among Mabs that cross-reacted with NAc MenB PS, and those that did not. However, the Mabs that cross-reacted with NAc MenB PS appeared to have the highest bactericidal activity (i.e., lowest concentrations needed to activate 50% killing [BC<sub>50</sub>]). Analysis of autoreactivity of the Mabs was performed using the CHP-134 human neuroblastoma (NB) cell line with and without neuraminidase (sialidase) treatment as a specificity control. Binding to this cell line was detected with several of the Mabs. Alternative approaches for measuring autoantibody activity are being used to confirm the pattern of reactivity. In conclusion, the murine anti-NPr MenB PS monoclonal antibodies described here are heterogeneous with respect to cross-reactivity with NAc MenB PS, their ability to bind to the NB cell line, and their ability to elicit complement-mediated bactericidal activity. Within the panel of Mabs there are examples of anti-NPr MenB PS Mabs that are bactericidal but do not cross-react with NAc MenB PS and do not show binding to the NB cell line. Presumably such antibodies could protect the host against the pathogen and pose no risk of eliciting autoimmune
disease. However, many of the anti-NPr MenB PS antibodies cross-reacted with native NAc MenB PS and also showed strong binding to the NB cell line. Although there is no evidence that the ability of an antibody to bind to host tissue will necessarily result in autoimmune disease, it will be a difficult task to prove that a conjugate vaccine that elicits such antibodies is safe to use in humans.

References

Immunogenicity of a meningococcal serogroup A and C conjugate vaccine in UK infants.

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Currently approximately 1500 cases of meningococcal infections are notified each year in England and Wales accounting for about one third of infections (1). Serogroup C vaccines have been developed from capsular polysaccharide but, unconjugated, these vaccines do not protect those under two years of age (2). Similar experience with the Haemophilus influenzae type b (Hib) native polysaccharide led to the development of the Hib conjugate vaccines, which have demonstrated enhanced immunogenicity in young infants and the capacity to induce immunological memory (3). Initial trials addressing the safety and immunogenicity in adults of a serogroup A and C conjugate vaccine demonstrated significant rises in antibody levels to both A and C polysaccharide and bactericidal antibody titers to serogroup C meningococci (4).

The most important role for an effective meningococcal conjugate vaccine is the protection of infants and children. Studies in infants in the Gambia with a serogroup A and C polysaccharide-conjugate vaccine have demonstrated high levels of serogroup C antibodies although the bactericidal activities of these antibodies were not measured (5).

In this study the immunogenicity of a serogroup A and C meningococcal polysaccharide-CRM197 conjugate vaccine was evaluated in 58 infants who received three doses at two, three and four months of age. Sera were tested for antibodies to the serogroup A and C capsular polysaccharide by enzyme-linked immunosorbent assay (ELISA) and bactericidal assays, against two serogroup C strains, using standardized protocols (6,7).

The pre-vaccination total immunoglobulin geometric mean titers (GMT) to anti-A and C polysaccharide antibodies were respectively 2.8 and 0.6 µg/ml rising to 21.5 and 38.5 µg/ml one month after the third dose and falling to 3.1 and 2.2 µg/ml by 14 months of age. Pre-vaccination serum bactericidal titers against two serogroup C meningococci were <1/4 in 49 out of 52 infants, rising to a GMT of 1/3082 one month post third dose and falling by 14 months of age to a GMT of 1/10. Thus this meningococcal conjugate vaccine proved to be immunogenic, inducing high levels of anti-C polysaccharide antibodies which were bactericidal in young infants. This is the first report of a significant antibody response to a meningococcal polysaccharide-protein conjugate vaccine in non-African infants and the first report of an effective bactericidal response in infants.

References


Evaluation of the innocuity of a group B meningococcal polysaccharide conjugate in hyperimmunized, pregnant cynomolus monkeys and their offspring

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Introduction. The poor immunogenicity of group B meningococcal polysaccharide has been suggested to result from its structural similarity with carbohydrate groups borne by the mammalian glycoprotein N-CAM (1). During feto-embryonic and early postnatal development, NCAM exhibits long polysialic acid (PSA) chains [α2-8 (NeuAc)n with n>8] which are recognized by B polysaccharide-specific antibodies whereas adult forms, with shorter chains, are negative (2). Several studies have shown that the expression of PSA on NCAM found, in particular, on neuroectodermally and mesodermally derived cells, modulates cell-cell interactions occurring during vertebrate organogenesis (3). Thus, it was hypothesized that pregnancy could be hindered or fetal development perturbated by antibodies cross-reacting with α 2-8 (NeuAc)n long chains. The conjugate we prepared for vaccine purpose is composed of the capsular polysaccharide of group B meningococcus, chemically modified (N-propionylated) and covalently linked to tetanus toxoid. The main objective of this study was to assess, in cynomolgus monkeys, whether hyperimmunization with the conjugate, resulting in high levels of maternal antibodies specific for N-propionylated B polysaccharide (B N-Pr), would affect gestation and/or the morphology and behavior of the offspring.

Study design. The study involved 39 adult female cynomolgus monkeys. One group of 24 was hyperimmunized (before mating) with the conjugate administered with Freund's complete adjuvant (FCA) and with booster injections of the same antigen with Freund's incomplete adjuvant (FIA) and then (after mating) with aluminum hydroxide (Al). One control group of 15 received the adjuvants. The study included several parts: reproduction study itself, characterization of the antibodies induced in females and transmitted to their offspring, behavioral tests on newborns, immunohistochemical and histological examinations on fetal tissues and histological studies of stillborns.

Results. The presence of antibodies specific for the vaccine antigen was monitored in the sera of females: IgG and IgM B N-Pr polysaccharide specific antibody titers were very high and sustained throughout the study (more than one year). Of the 11 hyperimmunized mothers getting full gestation, 8 were among those having the highest titers. Induced antibodies were bactericidal to group B meningococcus. Their ability to recognize polysialylated structures was also studied. Induced IgG recognized unmodified B polysaccharide far less than B N-Pr (about 1% cross-reactivity); the sera of 3 hyperimmunized females among the 11 getting a full gestation reacted strongly with PSA-NCAM structures in two highly sensitive in vitro tests.

The reproduction study showed no significant difference between the 2 groups of females in terms of fecundation, abortion and stillbirth rates:

fecundation rate: 75% for hyperimmunized group versus 87% for control group
abortion rate: 61% for hyperimmunized group versus 62% for control group
stillbirth rate: 43% for hyperimmunized group versus 67% for control group.

The analysis of antibodies specific to the polysaccharides, in the sera from fetuses and newborns, demonstrated that a large proportion of maternal IgG had been transmitted to them in utero. These passively acquired antibodies were only slightly bactericidal. Their levels decreased to reach a titer near the ELISA detection threshold, at 3 months of age. Few sera of infants obtained near delivery, recognized PSA-NCAM and only to a small extent.

The study conducted on 6 infants (2 from control mothers and 4 from hyperimmunized mothers), from 10 days of age until 6 months of age, included morphometric observations and neurobehavioral tests (4) to evaluate the neurologic development of these sucklings: neither constitutive abnormality nor behavioral difference were observed between infants born to control females and to hyperimmunized females.

Immunohistochemical investigations were conducted on tissues from 5 fetuses taken by caesarian about one month before the end of gestation, 4 of which were born to hyperimmunized females. Histological examinations provided additional data concerning these 5 fetuses and also involved the 8 stillborns, 3 of which were born to hyperimmunized mothers: no maternal antibodies linked to the polysialylated structures of fetal organs were found on immunohistochemical investigations, and histological examinations of fetuses and stillborns evidenced no particular lesions.

**Conclusion.** Within the limits of the number of cases observed in the present study and of the sensitivity of the methods used, it can be concluded that a high amount of antibodies specific for N-propionylated B polysaccharide, present during all the gestation in cynomolgus monkeys and transmitted to the fetuses had no harmful consequences on the development of the organs and nervous system of fetuses and sucklings.

**References.**
Safety and immunogenicity of an N-propionylated group B meningococcal polysaccharide conjugate vaccine in adult volunteers

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Introduction. Meningococcus is the second cause of bacterial meningitis after H. influenzae type b, serogroups B and C being predominant in industrialized countries. Although meningococcal groups A, C, Y and W135 capsular polysaccharide vaccines have been shown to be safe and effective, attempts to develop polysaccharide-based vaccines to prevent group B infections have remained unsuccessful. It has been shown that a chemically modified group B meningococcal polysaccharide conjugate is able to induce polysaccharide specific IgG and protection in mice (1,2). Thus, we developed a conjugate vaccine using the N-propionylated B polysaccharide (B N-Pr), the tetanus toxoid as carrier and Aluminum hydroxide (Al) as adjuvant.

Phase I study design. The safety and immunogenicity of the vaccine were evaluated in healthy male volunteers (age ranging from 19 to 27 years). Four escalating doses (1, 5, 25 and 50 µg of polysaccharide) were tested and injected intramuscularly into 3 individuals for 1 and for 5 µg and 5 individuals for 25 and for 50 µg. Volunteers received 3 injections at 4 weeks intervals. Sera were obtained before the first and one month after each injection. Safety studies involved the observation of local and systemic reactions and the evaluation of binding of the serum antibodies to α2,8-linked polisialic mammalian structures (polysialylated form of N-CAM named PSA-NCAM). The immunogenicity study included the determination of the amount and the functionality of the induced antibodies.

Results.

Local reactions observed were dose-related but not more severe than those usually produced by Al-adsorbed vaccines. No systemic reactions were recorded. No binding of serum antibodies (IgG and IgM) to purified PSA-NCAM or to tumor cells expressing PSA-NCAM was demonstrated. The serological analyses showed that all preimmune sera (16/16) contained IgM specific for B N-Pr and B (B PS) polysaccharides but no specific IgG.

Immunization with the conjugate elicited: a) an increase of the preexisting IgM titers; these titers sustained at least for one month after the last injection (mean seroconversion rate = 19 for B N-Pr and = 3 for B PS); b) B N-Pr specific IgG with both a dose and a booster effect (induced IgG were mostly from IgG1 subclass); c) no B polysaccharide specific IgG; d) an increase of anti-tetanus titers (mean seroconversion rate = 7, for the 2 highest dosages)
Functional activities of pre- and post-immunization sera were investigated. Bactericidal assays performed with human complement did not demonstrate any specific bactericidal activity whatever the day of blood sampling. However, high preimmune bactericidal titers were observed in the presence of baby rabbit complement, but immunization did not induce a significant increase of these titers. Purification of antibodies showed that IgM were responsible for most of this activity and that induced IgG were far less effective.

The following experiments were also performed on preimmune and post 3 sera from the 2 highest dosage groups: a) opsonophagocytosis using human PMN and human complement; b) Passive protection test in infant rats. No functional activity of the induced antibodies could be determined through these tests. However, the ability of post 3 antibodies to bind to group B meningococcus was demonstrated. On average, 100 % of B PS specific IgM, 83 % of B N-Pr PS specific IgM and 23 % of B N-Pr specific IgG bound to the bacteria.

Conclusion. These findings indicate that the conjugate is safe and immunogenic in human adults and able to induce an increase of preexisting B PS specific IgM titers and B N-Pr specific IgM and IgG.

Four hypotheses can be proposed to explain the apparent lack of function of induced antibodies: a) presence of high preimmune bactericidal titers (with baby rabbit complement), b) induction of too low levels of antibodies by the vaccine, c) lack of sensitivity of functional tests, and d) restriction of the functional tests used, to some aspects of the immune response.

References

Immunological activity of serogroup B meningococcal vaccine from natural complex of capsular polysaccharide and outer membrane proteins

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Antibody response of adult volunteers given a natural complex of capsular polysaccharide and outer membrane protein vaccine was studied by bactericidal assay (1). The vaccine used in these studies was prepared from Neisseria meningitidis group B serotype 2b strain 125 (B:2b:P1.2) (2), and contained 50 µg of group B polysaccharide and 57.7 µg of outer membrane protein per 0.5 ml dose when lyophilized vaccine was reconstituted with either aluminum hydroxide gel (concentration of AH 4.6 mg/ml) or with 0.9% NaCl.

The vaccine corresponded to WHO requirements (3) concerning sterility, pyrogenicity, and general toxicity. Volunteers at the age of 18-20 years were included in the study. Blood specimens were obtained from all volunteers before vaccination and four weeks after the second immunization. Only local reactions without systemic reactions were registered.

Bactericidal activity of sera was studied against three group B strains; B:2b:P1.2, B:2a:P1.2, and B:15:P1.7. Vaccine administered with and without AH induced bactericidal antibodies to all three strains. High antibody levels were detected after the second immunization when the AH gel was used. Eighty percent of individuals showed a four-fold or greater increase in bactericidal antibody titers to the homologous strain and 60% to the heterologous type 15 strain. The antibody response of adult volunteers who received the vaccine without adjuvant was less. Only 40% of individuals showed four-fold or greater increases in antibody titers to the homologous strain and 20% to the heterologous strain.

References

Cost-effectiveness analysis for routine immunization with a quadrivalent meningococcal polysaccharide (A,C,Y,W-135)- protein conjugate vaccine in the United States

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Background. Meningococcal disease is a leading cause of meningitis and septicemia among children less than five years old in the United States. The currently available meningococcal polysaccharide vaccine (A,C,Y,W-135) is poorly immunogenic in infants and young children making it unsuitable for use in routine infant immunization programs for the control of endemic meningococcal disease. The recent success in development of polysaccharide-protein conjugate vaccines against Haemophilus influenzae type b (Hib) disease has prompted development of vaccines using similar technology for Neisseria meningitidis. We evaluated the potential cost-effectiveness (C-E) of a quadrivalent meningococcal polysaccharide-protein conjugate vaccine (MenConj) (serogroups A, C, Y, W-135) when used as routine infant immunization in the United States.

Methods. We developed a C-E decision model, using the societal perspective, that compares the costs and benefits from routine infant immunization to the costs and benefits from the current situation where no routine infant immunization exists. Key estimates for meningococcal disease incidence, vaccination program, and costs associated with meningococcal disease are summarized below.

*Meningococcal disease incidence due to serogroups A, C, Y, W-135-- estimated birth cohort of 3,979,000 with a cumulative incidence between ages 6-59 months of 8.9 cases/100,000 [average incidence from CDC active surveillance in the United States between 1989-1995], a 8% case-fatality rate, and a 12% sequelae rate (1).

*Vaccination program-- 89% vaccination coverage, 90% vaccine efficacy, 4 doses of MenConj vaccine administered at ages 2, 4, 6, and 12-15 months of age in the same syringe as Hib conjugate vaccine, a vaccine cost of $4.76 per dose (the current public sector price of Hib conjugate vaccine), moderate and severe adverse reaction rates equivalent to those used for C-E models of Hib vaccines (0.002 and 0.000017) (2,3) and average costs per moderate and severe adverse reaction of $45 and $1,500, respectively.

*Costs associated with meningococcal disease-- direct costs include hospitalization for all patients ($13,431 per case). Long-term costs of sequelae were calculated with the same annual costs (adjusted to 1995 dollars) and duration used by the Institute of Medicine for a similar analysis of Hib vaccines (4). The discounted present value of lifetime costs per sequelae were $44,187 for a hearing deficit, $110,467 for a learning deficit, and $864,980 for an institutionalized patient with severe retardation. Indirect costs were calculated using estimates for lifetime productivity losses due to death or severe retardation ($964,490).
The costs to individuals and to health departments for chemoprophylaxis of contacts in response to sporadic cases of meningococcal disease and mass vaccination campaigns to control meningococcal disease outbreaks are not included.

Univariate sensitivity analyses (analyses where the estimate of one model parameter is varied from that used in the C-E model, with all other parameters remaining the same) were conducted on vaccine cost ($4.76 v. $2.80), number of doses in the vaccination regimen (4 v. 3), and disease incidence (8.91/100,000 v. 14.18/100,000).

We calculated three commonly used measures of the cost-effectiveness of vaccination: the cost per case averted, the cost per death averted, and the cost per life-year saved. Life-years lost were calculated by subtracting the average age of death from the average life expectancy. All costs and benefits were calculated in 1995 dollars, and future costs and benefits (including life-years saved) were discounted at a rate of 3%.

**Results.** In the C-E model, where no routine meningococcal vaccination exists, an estimated 355 cases, 28 deaths, and 843 years of life are lost annually due to meningococcal disease potentially preventable by a quadrivalent MenConj vaccine among children 6-59 months old, at a total estimated cost of $48.1 million. Routine infant immunization would be anticipated to prevent 284 cases, 23 deaths, and 675 years of life lost, annually. At a cost of $4.76/dose of MenConj vaccine, the costs per case averted, per death averted, and per life year saved are $98,146; $909,303; and $41,279 respectively. At a vaccine cost 2.80$/dose, the vaccine program results in net savings. If the same vaccine effectiveness can be accomplished with 3 doses, instead of 4 doses, of vaccine, the cost per case averted decreases by 60% to $38,787 per case averted. If the most recent estimate of disease incidence is used (14.18/100,000) [Source: CDC active surveillance in 1995], rather than the average of 7 years surveillance, the cost per case averted decreases by 85% to $9,419 per case averted.

**Discussion.** Meningococcal disease is a substantial economic and disease burden in the United States. The estimates of vaccine C-E from this analysis are likely to be conservative underestimates of the true C-E because the substantial costs to health departments in response to meningococcal clusters are not included. The C-E of MenConj vaccine in this analysis depends on some important assumptions. First, it must be administered in the same syringe with Hib conjugate vaccine (or other appropriate vaccine), thereby eliminating costs for additional visits or equipment to store and administer the vaccine. Second, we assumed that the vaccine will provide 90% protection for at least 4.5 years. Vaccine cost and the number of doses required to successfully immunize an infant strongly influence the C-E of routine MenConj immunization, as does the estimated disease incidence. Vaccine manufacturers can help optimize the C-E of MenConj vaccine by efforts to provide MenConj vaccine at cost equal to or less than that of Hib conjugate vaccine and in a formulation that allows it to be administered in the same syringe as Hib conjugate vaccine (or other appropriate vaccines). Herd immunity through decreased carriage of meningococci similar to what has been observed with use of the Hib conjugate vaccines may also improve the C-E of vaccination. While use of a quadrivalent MenConj vaccine against serogroups A, C, Y, W-135 in routine infant immunization programs is likely to have a substantial impact on endemic meningococcal disease, an effective serogroup B
meningococcal vaccine appropriate for use in infants is needed for comprehensive control of endemic meningococcal disease.

References

Bivalent A/C meningococcal conjugate vaccine in toddlers: persistence of antibodies and response to a polysaccharide vaccine booster

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We evaluated the immunologic memory elicited by a bivalent A/C meningococcal oligosaccharide conjugate vaccine (MOCV) (Chiron Biocine) compared to the licensed quadrivalent meningococcal polysaccharide vaccine (MPV). In an earlier study, 1 ninety 18-month-olds had been randomized to 3 groups and given 2 doses (2 months apart) of 1) MPV, 2) 5.5 µg MOCV, or 3) 11 µg MOCV. Following two doses, geometric mean antibody levels against group C meningococcus were twice as high in MOCV recipients as in MPV recipients, but antibody levels against group A were not significantly different. Serum bactericidal assays showed striking differences between the conjugate and polysaccharide vaccine groups, as MOCV induced much higher titers of bactericidal antibody against both serogroups. We concluded that the immune response induced by the meningococcal conjugate vaccine was qualitatively different from that induced by the polysaccharide vaccine, and that the antibodies it elicited provide greater functional activity. In this follow-up study, serum specimens were obtained from 25 children in each group 1 year after the 2nd dose. A booster dose of MPV was offered to all children, and 11-14 subjects in each group accepted. Another serum specimen was obtained 1 month after the booster.

Group A meningococcal ELISA antibodies. Geometric mean antibody titers (GMT) were 22.7 µg/ml and 21.2 µg/ml in MOCV and MPV recipients, respectively, after the 2 dose primary series (p = 0.7). Antibody levels fell markedly in the year following vaccination, and did not differ significantly between MOCV and MPV recipients (4.5 µg/ml vs. 6.7 µg/ml; p = 0.26). Following the booster dose of polysaccharide vaccine, antibody levels increased in all children and the GMT was 3-fold higher in conjugate vaccine recipients (78.0 µg/ml vs. 22.2 µg/ml; p < 0.006). In contrast to children who had been given MPV as their primary series, in children initially given MOCV the GMT was significantly higher after the polysaccharide booster than it was after the 2 dose primary series (p < 0.0001).

Group C meningococcal ELISA antibodies. After the initial 2 vaccine doses, the GMT was significantly higher in MOCV recipients compared with MPV recipients (16.7 µg/ml vs. 8.3 µg/ml; p < 0.001). However, antibody levels against group C declined sharply in the year after vaccination, and did not differ significantly between groups (1.5 µg/ml vs. 1.4 µg/ml; p = 0.94). After the polysaccharide booster, antibody levels increased in all vaccinees, but the GMT was more than 5-fold higher in children given MOCV compared with those given MPV (29.9 µg/ml vs. 5.3 µg/ml; p < 0.0001). Furthermore, unlike children initially given MPV, MOCV recipients had higher antibody levels after the booster than they did after their primary series (p < 0.004).
**Group A serum bactericidal activity.** After the primary vaccine series, the geometric mean serum bactericidal titer (SBT) was significantly higher in conjugate vaccine recipients (755.6 vs. 37.6; p < 0.0001). One year later, serum bactericidal activity did not differ significantly between MOCV and MPV recipients (13.5 vs. 7.1; p = 0.22). Whereas all conjugate vaccine recipients had serum bactericidal titers $\geq 128$ after their primary vaccinations, 38% had no detectable bactericidal activity a year later. However, after the polysaccharide booster, the geometric mean SBT was about 16-fold higher in conjugate vaccine recipients compared with polysaccharide vaccine recipients (1673.1 vs. 107.6; p < 0.0001) After the booster, all children initially given MOCV had detectable serum bactericidal activity and 92% had titers $\geq 1024$, whereas 17% of children initially given MPV had no detectable serum bactericidal activity.

**Group C serum bactericidal activity.** After the primary vaccine series, the geometric mean SBT was significantly higher in MOCV recipients compared with MPV recipients (3197.9 vs. 11.4; p < 0.0001) Serum bactericidal activity fell sharply in the following year. Although the geometric mean SBT remained significantly higher in conjugate vaccine recipients (101.6 vs. 4.5; p < 0.0001), 21% of children initially given MOCV no longer had detectable serum bactericidal activity. After the polysaccharide booster, the geometric mean SBT was almost 1000-fold higher in children given MOCV compared with those given MPV (6502.1 vs. 7.1; p < 0.0001). All children given the conjugate vaccine had bactericidal titers $\geq 1024$, whereas 83% of polysaccharide vaccine recipients had no detectable serum bactericidal activity.

As has been shown with *Haemophilus influenzae* type b2 and pneumococcal3 conjugate vaccines, this meningococcal conjugate vaccine primes for memory antibody responses. Compared with the licensed polysaccharide vaccine, the antibodies it elicits have much greater functional activity against both serogroup A and serogroup C meningococcal polysaccharides. It offers the potential of providing durable protection against these important pathogens.

**Acknowledgement.** Funded by NIAID.

**References**

Analytical methods for the quality control of *Neisseria meningitidis C* polysaccharide.

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**Introduction.** The problem of conformational stability control is an important factor. A detailed analysis of conformational changes requires the use of exact, precise and sensitive techniques (1). Using HPLC and NMR methods gives a very interesting information about molecular size and primary structure of polysaccharide C and more accurate than others methods. Physical parameters had been reported, such as molecular weight, radius of gyration, diffusion coefficient, viscosity, light scattering for group C meningococcal (2). In this paper, we evaluated some methods for the quality control and stability studies.

**Materials and methods.** Serogroup C meningococcal polysaccharide was purified from *Neisseria meningitidis* and was stored at -70°C.

Chromatography: The samples employed were dissolved in distilled water. Molecular size was determined by gel filtration on Sepharose 4B (Pharmacia Fine Chemicals) using 0.2 M ammonium acetate (3) and by HPLC on TSK G-3000 column (4).

Viscosimetry: The polysaccharides (1 mg N-acetylneuraminic acid/ml) were used to measure the rheology properties when they are stored at some temperatures. Reduced viscosity was performed on Ubbelohde viscometer.

Optical rotatory dispersion. Optical rotations were measured by Polartronic universal polarimeter with halogens lamps and sodium filter.

NMR spectroscopy: Samples were dried in vacuum over P2O5 and then dissolved in deuterium oxide in a 5 mm NMR tube. Spectra were obtained at 250 MHz on a Bruker spectrometer (5).

D.S.C.: A Mettler differential scanning calorimeter was used, cooling rate 5°C min⁻¹, hating rate 5°C min⁻¹.

**Results.** Molecular weight determination of polysaccharide on HPLC has a fitting precision (variation coefficient below 2 %). The differences with chromatography on Sepharose 4B increased 40 %. The viscosity to different polysaccharide lots (stored at -70°C) was 3.43 ± 0.54 ml . mg⁻¹. The value of specific optical rotation indicated - 5.32 ± 1.3°. g⁻¹. cm⁻². The irreversible conformational changes of polysaccharide produced by temperature to determine changes in the physical parameters. NMR spectra for the type C polysaccharides from different lots showed a similar pattern at Jones and Currie’s spectra. The most important region is containing methyl resonance from O-acetyl groups. Calorimetric measurements gave a glass transition temperature of approximately -12°C and crystallization temperature was nearly -35°C.
Discussion. The dependence diffusion coefficients of molecule’s viscosity impose difficulties in partition coefficients values. HPLC would partially resolve the problems quicker and with more accuracy. Some conformational events in subgroup C polysaccharide would be characterized by optical rotation and viscosity studies. The similar NMR spectra between production lots shown homogeneity in purification processes and a good stability at -70°C. In the thermograms obtained, it is evident that a crystallized state can protect polysaccharide against degradation, in the stored condition.

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Long-term follow-up of late complement component deficient patients vaccinated with meningococcal polysaccharide vaccine: Antibody persistence and efficacy of vaccination.

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Of 45 Russian patients with late complement component deficiency (LCCD) who experienced one-to-five meningococcal infections, thirty-three were immunized with meningococcal polysaccharide vaccine (A+C+W135+Y) and followed for one to five years. Their immune status was normal before vaccination (1). The pre-vaccination levels of antibodies to A, C, W135 and Y polysaccharides were slightly, but not significantly, higher in the group of LCCD patients, than in the control group of 33 vaccinees without complement deficiency and meningococcal infections.

Total and immunoglobulin class specific concentration of antibodies to meningococcal capsular polysaccharides in sera of LCCD patients increased significantly one month after vaccination and remained stable for 1 year. Total Ig levels to A, C, W135 and Y were increased 5, 15, 15 and 16 times, respectively. The specific antibody declined in the next 2-3 years, but remained at least two fold above the concentration in preimmunization sera. The proportion of specific IgA to IgA+IgM+IgG was about 20% before vaccination, and either remained stable (to group A polysaccharide) or decreased (to other polysaccharides) after vaccination. Thus vaccination maintained or increased the prevalence of Ig classes, having complement-activating and opsonophagocytic functions. The kinetic and quality of antibody response did not differ in LCCD vaccinees and control group (2).

Revaccination of 12 patients 3 years after the first dose restored the total antibody concentrations to those observed one year after the first vaccination. The increase was mainly caused by specific IgG, which consisted 80-90% of total specific Ig after revaccination. All patients developed a significant antibody response to all polysaccharides after revaccination, whereas after the first dose one fifth of the patients had only a weak response to some of the polysaccharides.

Six new episodes of meningococcal infection in four patients developed in the group of 33 vaccinees; one of these cases occurred after revaccination. Six episodes in six patients developed in the same time in the group of 12 non-vaccinated LCCD persons. Survival analysis demonstrated that the risk to contract meningococcal disease decreased significantly for vaccinees (0.04 episodes /individual /year) in comparison to non-vaccinees (0.15). The interval between consecutive infections was prolonged from 3.6 years in the non-vaccinated group to more than 6 years (p < 0.02, Kaplan-Meier test) in the vaccinated group. Data from a historical control (same patients followed for ten years before vaccination) was comparable to the later
data of non-vaccinated LCCD persons; the interval between the consecutive infections was 10 years and the risk of disease was 0.15 episodes /individual /year in both groups.

Two LCCD vaccinees experienced two episodes of disease after vaccination. This statistically hardly probable event (p < 0.07) indicated that some individual properties might have caused the vaccination failure in these cases. However, no differences in immune response were found between the LCCD vaccinees, who were (N = 4) and were not (N = 29) newly infected.

Unfortunately no clinical isolates of meningococci were obtained from ten newly infected patients; the clinical diagnosis was confirmed by microscopy in 7 cases and PCR in 5 cases. No significant increase of antibody level to group A, C, W135 and Y polysaccharides was detected after five new episodes of meningococcal disease in vaccinees suggesting that post-vaccination infections were caused by group B meningococci. After one episode the level of antibody to group C polysaccharide was increased from a relatively low level of 4.8 mg/l to 72 mg/l, suggesting group C infection. The antibody concentrations after the disease of non-vaccinees were not studied. In 1980-1990 Russian LCCD patients were infected by meningococci of group A (3 isolates), B (3 isolates), C (1 isolates) and unidentified serogroup (3 isolates).

In conclusion, vaccination with of A+C+W+Y polysaccharide vaccine seems to decrease the risk for meningococcal infection in LCCD patients even in a situation when group B meningococci are most frequent serogroup in general (3). This protection might be caused by an increased killing capacity of neutrophils (4).

References

NMR analysis of meningococcus type A and C polysaccharide antigens: patterns of O-acetylation

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Biocine SpA has developed conjugate vaccines against meningococcal serotypes A and C microorganisms. The production of these glycoconjugate vaccines all involve generation of the suitable oligosaccharides by fragmentation of the capsular polysaccharide. This procedure and the following steps of oligosaccharide activation and subsequent conjugation to the carrier protein (CRM₁₉₇) can be monitored by colorimetric and other traditional methods of analysis. However, recent advances in analytical technology permit the structure of these complex immunogens and precursors to be evaluated by physicochemical methods, particularly by NMR analysis. As a starting point in our characterization studies, we have examined the type A and C polysaccharide antigens and will present our data here. The ¹H, ¹³C and ³¹P 1D spectra provide fingerprints which can be used to show the identity and purity of different polysaccharide lots. The unambiguous assignment of these resonances and hence confirmation of the molecular structures follows from the use of 2D NMR homonuclear and heteronuclear correlation spectroscopy (1).

The MenC polysaccharide consists of a simple homopolymer of 2,9-α-linked sialic acid, however, the NMR spectra are complicated due to the presence of O-acetyl groups (2). The O-acetyl ester groups are alkali labile but can migrate spontaneously under physiological conditions. Most studies of O-acetyl migration have been performed by NMR analysis applied to simple systems such as the sialic acid monomer (3). The data show that acetyl groups at O-7 and O-8 migrate spontaneously to O-9. T₁/₂ for the O-7 acetyl migration to O-9 is 4-8h at physiological pH and temperature, whereas that from O-8 to O-9 is too rapid for measurement.

For the MenC polysaccharide, elucidation of this spectral complexity is hampered by the fact that acetyl migration occurs in solution and thus may change during the actual NMR analysis. Not surprisingly, most of the proton and carbon NMR data published are for the de-O-acetylated polysaccharide (4-6). A ¹³C study of the native polysaccharide with assignments based on inspection, showed that O-acetylation is at O-7 and/or O-8 (4). We have confirmed this from our detailed 2D NMR examination of our MenC lots. We were able to make assignments of the major ¹H and ¹³C resonances of the different spin systems and so are able to interpret the spectra of our batches as well as those published. The amount and position of O-acetylation can be determined most easily from the intensity of the different acetyl signals and recording the spectra with time shows that acetyl migration occurs from O-8 to O-7. This reaches an equilibrium which appears to be 50% complete after 7 days at room temperature (7). We suspect that the acetyl group is initially solely on O-8 and begins to migrate to the more stable position O-7 during isolation of the antigen and continues when the polysaccharide is in solution. This means that the spectrum obtained depends on the history and preparation of the actual sample, as well
as the conditions under which the spectrum is recorded. Different acetylation is manifested by signals near δ5 due to H7 and H8 as well as different H3 and CH$_3$CON signals so that spectra can appear different although they are still of the MenC polysaccharide. This accounts for the large spectral differences observed between preparations of the same antigen from different manufacturers (2). It is not yet known how acetylation in different positions affects immunogenicity, as most investigations of the relationship between O-acetylation and immunogenicity have been restricted to the presence or absence of O-acetyl groups (8, 9).

In the case of the MenA polysaccharide, full proton, carbon and phosphorus assignments were made by use of 2D homo- and heteronuclear NMR experiments. The PS was reported to be →6)-α-ManNAc-(1-OPO$_2$ → containing approximately 70% O-Ac at O-3 (10). These assignments were made by use of 1D 13C NMR, but our 2D NMR experiments clearly reveal the presence of at least 3 spin systems: unacetylated (29%), Ac on O-3 (66%) as well as on O-4 (5%). The TOCSY diagram showed that the mannosyl H2 resonance is very useful for the “fingerprinting” of the different spin systems. With this knowledge, it is possible to interpret the spectra published for other manufacturer’s vaccines (2); this reveals acetylation on O-3 as well as O-4.

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An improved method for meningococcus C polysaccharide purification

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During the past decade vaccines of Neisseria meningitidis group A, C, W135 and Z have been developed. These vaccines are composed of purified polysaccharides which are the main component of bacterial capsule. N. meningitidis capsular polysaccharide purification method currently in use was first described in 1969 by Gotschlich [1]. By this method, polysaccharide together with protein, nucleic acids and LPS contaminants are precipitated with 0.1% Cetavlon followed by resuspension in 1M CaCl$_2$. Nucleic acids are eliminated by precipitation in 25% ethyl alcohol, proteins are extracted by 45% phenol and LPS are pelleted by ultracentrifugation at 100,000g for 4 hrs. This process has two inconvenient steps for large scale production: phenol is a very corrosive reagent and therefore contaminant protein elimination by phenol extraction should be substituted and ultracentrifugation step is expensive for large scale production since many ultracentrifuges are needed.

In the method established at Instituto Butantan for group C polysaccharide purification, the initial steps were made as already described [1] and two steps of this procedure have been modified: removal of protein and LPS.

Contaminant protein was removed by proteinase digestion using a mixture of three proteinases: proteinase K, nagarse and trypsin. After 25% of ethyl alcohol precipitation and nucleic acid removal by centrifugation, the supernatant was precipitated with ethyl alcohol to 80%. The precipitated PS was resuspended in 20mM Tris-HCl buffer pH 8.5. This solution was incubated overnight at room temperature with 5 mg of each proteinases (for 40L of fermentor), and this treatment was repeated for further 4 hours.

LPS forms a high molecular weight complex which is efficiently pelleted by ultracentrifugation [1]. Detergents such deoxycholate (DOC) are able to disaggregate the complexed LPS to a low molecular weight monomers. [2]. Tangential ultrafiltration on hollow fiber 100 kDa cutoff in buffer containing DOC was used instead ultracentrifugation. Extensive diafiltration on the 100 kDa cutoff hollow fiber (AMICON) in Tris-HCl buffer containing 0.5% DOC was able to eliminate LPS as well as low molecular weight protein resulted by proteinases action. The solution was washed in hollow fiber with five separate volumes of 20 mM of Tris-HCl buffer pH 8.5 containing 0.5% DOC, five separate volumes of the same buffer without DOC and three separate volumes of water.

The resulted purified polysaccharide has around of 2% protein and 1.2% nucleic acid. The polysaccharide molecular weigh determined in Sepharose 4B column showed a $K_D$ around 0.3 and it passed in the pyrogen test in rabbit according to WHO. The polysaccharide recovery using this process was around 50%.
This process is easily suitable for scale-up due to its easiness. The great advantage of the hollow fiber use is the fact that large volumes for instance, a scale up of 40 to 400 L fermentation can be processed at the same time and by the same way.

(Supported by FAPESP (Proc. 94/3069-9) and Secretaria de Saúde do Estado de S. Paulo.)

References

Efficacy of *Neisseria meningitidis* serogroup A/C polysaccharide vaccine among children in Mongolia


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**Background.** During winter 1993-94, an increased number of meningitis cases were reported to the Mongolian Ministry of Health (MOH); cultures of blood and cerebrospinal fluid revealed *Neisseria meningitidis* serogroup A. Eight strains were subtyped by multilocus enzyme electrophoresis and found to belong to the III-1 clonal complex; strains within this complex have caused recent epidemics in the Middle East and Africa (1). From January-April 1994, a total of 1,754 meningitis cases resulting in 122 deaths were reported to the MOH; the country-wide attack rate was 80 case/100,000 population. The capital city of Ulaan Baatar reported 1070 cases during January-April 1994 and had an attack rate of 179/100,000, the highest among Mongolia’s provinces. In response, the MOH organized a campaign to vaccinate children 2-18 years of age in Ulaan Baatar with A/C meningococcal vaccine (France, Pasteur-Merieux) in November 1994.

**Methods.** During June 1995 we conducted a retrospective case-control study to estimate vaccine efficacy and identify risk factors for disease. Case-patients ≤ 18 years old who had positive sterile-site cultures and a systematically selected group of other meningitis case-patients were identified for enrollment. Three controls matched by neighborhood and age were recruited for each case-patient. We collected information on exposures and vaccine history using a standardized questionnaire and reviewed written records to confirm vaccination status. To estimate the impact of the vaccination campaign, we compared cases of meningitis reported to the MOH from January-April 1995 to those received during the same period in 1994.

**Results.** In all, 85 cases and 255 controls were enrolled (32 case-patients < 2 years of age and 53 cases 2-18 years); 13 case-patients (15.3%) had culture-confirmed *N. meningitidis* serogroup A infections. We identified written confirmation of vaccination for 92% of cases and controls who reported receiving the vaccine. Using logistic regression, estimated vaccine efficacy for those 2-18 years of age was 91.9% (95% CI 76.3-97.2%). Among participants 2-4 years of age, estimated vaccine efficacy was 92.6% (95% CI 62.5-98.5%). Analysis limited only to those with written confirmation of vaccination showed the same point estimates of vaccine efficacy. In addition to vaccine efficacy, factors independently associated with disease included maternal educational level of secondary school or less for those 2-18 years of age (OR 4.0, 95% CI 1.3-11.8); living in Ulaan Baatar for at least 2 years reduced risk of disease (OR 0.4, 95% CI 0.1-1.1, p = .08). No significant risk factors for disease were identified among study participants < 2 years old. The incidence of meningitis in Ulaan Baatar decreased to 74 cases/100,000 during January-April 1995. The most dramatic decline occurred among those of vaccination age, where the incidence decreased 74% from 359 cases/100,000 in 1994 to 92 cases/100,000 in 1995. In contrast, the incidence of disease increase by 10.2% and 31.1%, respectively, among those <2 years and >18 years in Ulaan Baatar. Assuming a similar rate increase would have occurred
among those 2-18 years old without immunization, an estimated 555-730 cases were prevented by the vaccination campaign. Outside the capital, 17 of 21 provinces had higher meningitis attack rates in 1995 compared to 1994.

Conclusions. The results indicate that the *N. meningitidis* serogroup A polysaccharide vaccine is highly effective, even among those 2-4 years of age. This finding confirms apparent efficacy reported in earlier clinical trials among children (2,3). The estimated vaccine efficacy of 92% is similar to that previously found among military recruits (4). In response to the increasing incidence of disease in provinces outside the capital in 1995 and the high attack rate among infants, the MOH conducted a countrywide immunization campaign in Fall 1995 for children 2-18 years old and for infants between 6 months and 2 years of age in Ulaan Baatar.

References:
