CLINICAL MICROBIOLOGY REVIEWS, Apr. 1989, p. S134–S138
0893-8512/89/0SS134-05$02.00/0

Vol. 2, Suppl.

Vaccines for Prevention of Meningococcal Disease

CARL E. FRASCH

Division of Bacterial Products, Center for Biologics Evaluation and Research, Bethesda, Maryland 20892

Neisseria meningitidis is the second leading cause of bacterial meningitis in the United States and is the leading cause in many other countries. In the United States there are 3,000 to 4,000 cases of bacterial meningitis per year, for an incidence of about 1/100,000; *N. meningitidis* is responsible for a number of outbreaks each year. Control of these outbreaks and the severe epidemics that have occurred in many countries required the development of vaccines to prevent meningococcal meningitis.

N. meningitidis is divided into 12 serogroups based on the immunological specificity of their capsular polysaccharides (PSs): A, B, C, H, I, K, L, X, Y, Z, 29E, and W135 (35). Approximately 90% of meningococcal disease worldwide is caused by serogroups A, B, and C. Group A is now rare in the United States, but it is the major meningococcal pathogen in the "meningitis belt" of Africa and a number of Asian countries. In the United States, groups B and C are currently responsible for about 50 and 35% of meningococcal disease, respectively, with groups Y and W135 accounting for the remainder.

For epidemiological purposes, meningococcal strains are now identified by their serogroup, serotype, and subtype, e.g., B:15:P1.16. The disease-associated serogroups have been subdivided into over 20 different serotypes (12) based upon immunological differences in the class 2 and class 3 major outer membrane proteins (OMPs). These proteins are the meningococcal porins, having molecular weights, as estimated on sodium dodecyl sulfate-polyacrylamide gels, of between 34,000 and 40,000. The strains are further subtyped by the immunological specificity of the approximately 46-kilodalton class 1 OMPs. Class 1 proteins are shared by a number of different serotypes, and approximately 10 class 1 subtypes have been identified to date. As will be shown below, both class 1 and class 2 proteins have importance as potential vaccine candidates.

Protection against meningococcal disease has been correlated with the presence of bactericidal antibodies (8). The peak incidence of disease occurs in children under 1 year of age; as a group, they have few or no bactericidal antibodies. In addition, the high susceptibility of individuals with an inherited deficiency of one of the terminal complement components (C5, C6, C7, or C8) for invasive meningococcal disease strongly implicates the importance of bactericidal activity in host defense against these organisms (30). For these reasons, measurement of the bactericidal activity in serum provides an accurate indication of the resistance of an individual to meningococcal disease.

The development and clinical evaluation of meningococcal PS vaccines have been reviewed previously (8, 13, 31), as have the initial studies of the use of protein vaccines for group B disease (8). The present review will therefore concentrate on recent studies, recommendations for use of the PS vaccines, and the current status of a group B vaccine.

PS VACCINES

Immunization against *N. meningitidis* became a reality with the accomplishments of Gotschlich et al. in the late 1960s (14). The capsular PS vaccines developed by Gotschlich et al. were among the first chemically pure bacterial vaccines. The group C vaccine was used to prevent the severe outbreaks of group C meningococcal disease in the U.S. armed forces.

The molecular weight of the PS has been correlated with its immunogenicity (15). In early attempts in the 1940s to prepare purified group A meningococcal PS vaccines, the PS was found to be almost nonimmunogenic (20). Later studies by Gotschlich et al. (15) showed that the early failures were due to degradation of the PS. Hence, the PSs must be of high molecular weight as measured by gel filtration on Sepharose 4B or 2B to be included in the vaccine.

Group A PS

Efficacy of the group A vaccine was shown through a series of field trails in Africa and Finland in the early 1970s (26, 38). These trials indicated that the PS was approximately 90% effective in controlling epidemic group A disease. Protection in the Finnish trial began at 6 months of age (26). The duration of protection in the epidemic and hyperendemic areas of Africa was short in young children (28). Reingold et al. studied the duration of protection in young people, ranging from 6-month-old children to university-age students, against clinical group A meningococcal disease in Burkina Faso, Upper Volta (28). The individuals were vaccinated with an A/C bivalent vaccine. By using case-control studies, the efficacy was found to be 100, 74, and 67% for 1, 2, and 3 years after vaccination in individuals over 4 years of age at immunization, compared with 85, 52, and 8%, respectively, for those under 4 years of age. The rapid fall in protection in the younger children is probably related to the poorer response of young children and to the observation that malaria, endemic in western Africa, interferes both with the immune response to the PSs and with the persistence of antibodies (39).

The decline in protection has been correlated with a decline in group A PS antibodies. Kayhty et al. (21) found that the antibody levels in children who were vaccinated at less than 4 years of age were not different from those in unvaccinated controls 3 years postimmunization.

Group C PS

The group C PS was first shown to be efficacious in U.S. military recruits (1, 31), in whom it virtually eliminated group C meningococcal disease. The group C PS was later found to be effective in preventing disease in children over 2 years of age in an efficacy trial in Brazil (31). The duration of protection was not examined in the Brazilian trial.

The group C PS is a homopolymer of sialic acid-linked α2-9 and is found in two variant forms. Approximately 15% of group C clinical isolates elaborate an *O*-acetyl-negative PS, and the remainder elaborate an *O*-acetyl-positive polysaccharide. Studies were conducted in children and infants to compare the immunogenicity of the two variant PSs (27). Each of the PSs stimulated equivalent responses. The current PS vaccine contains the *O*-acetyl-positive PS.

Recent studies have been conducted to examine the antibody response of young children and infants to the ACYW135 tetravalent vaccine (22, 27). Lepow et al. (22) found that a greater percentage of children between 2 and 5 years of age responded to the Y and W135 PSs than responded to the A and C PSs. An 80% or better response was found to all four PSs in children aged 9 years or older. A problem with the PS vaccine in children aged 8 years and under was the rapid fall in antibody levels, 70 to 80% 1 year after vaccination.

Peltola et al. (27) examined the immune response of infants aged 6 to 24 months to the tetravalent vaccine given at 30 μg per PS. Children under 1 year of age at immunization received a second injection 3 months later. Over 90% of the children responded with bactericidal antibodies to group A. The response to the other PSs was more age dependent, with 80 to 100% of the 18- to 23-month-old children and 40 to 50% of the younger children responding to the PSs. Children under 1 year of age had a rapid decline in bactericidal antibodies, and 1 year later, levels were not different from those in nonvaccinated children of the same age. Peltola et al. recommended that children under 1 year receive two injections 3 months apart. They thought that routine immunization would be justified if an effective group B vaccine component could be added.

Recommendations

The Immunization Practices Advisory Committee of the U.S. Public Health Service has made recommendations for the use of the meningococcal PS vaccines (5). The A, C, AC, and ACYW135 PS formulations are currently licensed in the United States, and recommended immunization is a single 0.5-ml intramuscular injection of vaccine containing 50 μg of each PS. The vaccine is presently available in the United States from Connaught Laboratories and in Europe from Smith Kline-RIT and Institute Merieux.

Routine vaccination of the civilian population in industrialized countries is not currently recommended, because (i) the risk of infection is low, (ii) a vaccine against group B is not available, and (iii) most of the endemic disease occurs in young children. Vaccination is advised to control outbreaks due to meningococcal serogroups covered by the vaccine. Routine vaccination is recommended for travelers to countries recognized as having hyperendemic with periodic epidemic meningococcal disease, such as the meningitis belt of Africa, or recent epidemic disease, such as Nepal and Saudi Arabia.

Vaccination of individuals with a deficiency in one of the terminal complement components or with properdin deficiency may be effective in preventing disease. Patients with deficiencies in C5, C6, C7, or C8 have a prolonged susceptibility to meningococcal disease and often develop the disease later in life; the median age at the first episode is 17 years (30). The low mortality rate (4%) associated with such infections suggests that the presence of antibodies that can promote opsonophagocytosis is important in such individuals. Therefore, it follows that vaccination could provide some protection.

Normal properdin function appears to be necessary for resistance to meningococcal disease. Properdin promotes bacterial killing through activation of the alternative pathway by stabilizing the C3 convertase, C3bBb (33). Many individuals with meningococcal disease associated with properdin deficiency are young children, who would not be expected to have developed protective antibodies (33). Vaccination of properdin-deficient individuals with the meningococcal PS vaccines has been shown to induce antibodies that cause meningococcal killing through the classical complement pathway (6).

Conjugate Vaccines

For meningococcal PS vaccines to elicit antibodies in young children, they will have to be conjugated to protein carriers. The duration of protection in young children is relatively short, and the antibody response is not boostable as is the case with T-cell-dependent antigens. Meningococcal A, B, and C PSs and oligosaccharides have been covalently attached to tetanus toxoid (2, 3, 18). Jennings and Lugowski (18) produced conjugates of the A, B, and C PSs by periodate oxidation and direct attachment through the lysines in the tetanus toxoid. In addition, they prepared oligosaccharides from the group C PS and linked them through a spacer molecule to bovine serum albumin. These conjugates were used to hyperimmunize mice. All except the B conjugate were highly immunogenic. Beuvery et al. (2, 3) produced group A and group C PS conjugates by attachment to tetanus toxoid. These conjugates were compared with the native PSs for their immunogenicity in mice. The native PSs were essentially nonimmunogenic when administered in two doses 10 weeks apart. In contrast, a single injection of the conjugates elicited high antibody levels. A second injection at week 10 elicited a booster response, typical of a T-cell-dependent response. Adsorption of the conjugates to aluminum phosphate significantly increased the primary immune response, but failed to have an effect on the booster response (2, 3). Thus, conjugation of the A and C PSs to a protein carrier converted the PSs from T-cell-independent to T-cell-dependent antigens.

Meningococcal PS-protein conjugates have not been evaluated to date in humans. However, extensive human studies with *Haemophilus influenzae* type b PS conjugates indicate that conjugates are safe and much more immunogenic in young children than the native PS is (16). A second injection of an *H. influenzae* type b conjugate or of the native PS elicits a strong booster response (16). Whereas the type b PS was not effective in children under 18 to 24 months of age, a diphtheria toxoid conjugate proved to be highly efficacious in infants under 1 year of age (7). Meningococcal PS conjugates may also prove to be highly immunogenic in young infants.

GROUP B MENINGOCOCCAL VACCINES

Group B PS

Although the group B PS, a homopolymer of α2-8-linked sialic acid, appears the logical choice for production of a group B meningococcal vaccine, only limited success has been achieved in animal studies (19, 24). The native PS induces only a transient immunoglobulin M (IgM) response in humans (40). A number of possible explanations have been given for the poor immunogenicity, including sensitivity to neuraminidases and the similarity of the PS to sialic acid moieties in human tissues.

Moreno et al. have attempted to improve the immunogenicity of the group B PS by noncovalently complexing it to OMPs (24) and by adsorbing the complexed vaccine to aluminum hydroxide (25). These vaccines were used to immunize mice and then compared with vaccination with the pure PS. Although the PS was nonimmunogenic, both of the other formulations induced transient increases in IgM anti-

bodies that peaked on day 7. A similar aluminum hydroxide serotype protein group B PS vaccine failed to stimulate measurable increases in group B PS antibodies in human studies (11).

Another approach to preparing immunogenic group B PS vaccines is to covalently link the PS to a protein carrier or to chemically modify the PS. The group B PS conjugates were essentially nonimmunogenic. Jennings et al. made a number of chemical modifications to the PS (19). The only modification that did not abrogate the ability of group B-specific antibodies to recognize the PS was removal of the *N*-acetyl groups and substitution of *N*-propionyl groups.

The N-propionylated PS and its tetanus toxoid conjugate were used to immunize mice by using Freund complete adjuvant in a series of three injections (19). The altered PS alone failed to induce group B PS-reactive antibodies. In contrast, two injections of the conjugate induced antibody levels substantially above background, and the third injection gave a good booster response. Interestingly, the antibody response was mostly IgG. These antibodies were shown to be bactericidal against group B strains of different serotypes (17) but not against group A, C, or W135 strains. Quantitative precipitin experiments indicated that about half of the antibodies induced to the N-propionylated PS were specific for the altered PS (17). Unexpectedly, absorption of the sera with only the N-propionylated PS removed the group B bactericidal antibodies, even though the native PS absorbed all radioimmunoassay-reactive antibodies. Thus, the N-propionylated group B PS mimics a bactericidal epitope on the group B organism. Additional studies of the nature of this epitope are important, and limited adult human studies seem warranted.

Important OMPs

A very promising approach to the development of an effective group B meningococcal vaccine is the use of lipopolysaccharide-depleted OMP vaccines. Such vaccines are prepared from outer membrane fragments depleted of lipopolysaccharide by detergent treatment. The detergent is removed, and the final vaccine is formulated to also contain one or more meningococcal PSs to improve its solubility and immunogenicity.

Meningococcal outer membranes contain a number of major proteins, and most of these proteins are included among those designated as the class 1, 2, 3, 4, and 5 proteins (34). These proteins are often present in the OMP vaccines, which induce bactericidal antibodies in both animals and humans. Studies were therefore done to help define the OMPs responsible for the induction of protective antibodies (4, 32). Saukkonen et al. (32) have used their recently developed infant rat model for meningococcal infection to evaluate the protection afforded by monoclonal antibodies to the class 1 and 3 OMPs. Antibodies against both classes were bactericidal, but only the anti-class 1 protein monoclonal antibodies protected against both blood and cerebrospinal fluid infection by the homologous subtype. In similar studies, Brodeur et al. (4) examined monoclonal antibodies against OMP classes 1, 2, and 5 prepared against a serotype 2b strain for their ability to protect in a mouse model; mucin and hemoglobin were used to achieve infection. In this model none of the monoclonal antibodies prevented bacteremia, and only the class 2 serotype 2b-specific monoclonal antibody significantly reduced lethality. The passive protection studies with monoclonal antibodies, taken as a whole, indicate that the class 1 proteins are strong candidates for an OMP vaccine. These proteins are serosubtype proteins and are shared among meningococcal strains to a greater extent than the class 2 and 3 proteins are.

The antigen specificity of the adult response to a serotype 2a OMP vaccine was examined by immunoblotting (36, 37). Most individuals responded to some of the high-molecular-weight proteins and to the class 1 and 5 proteins. It was particularly noteworthy that those who responded with high bactericidal titers had high antibody levels to the P1.2 class 1 protein. The subclass response was primarily IgG1 and IgG3 (37). The class 1 protein is therefore an important antigen for inclusion in an OMP vaccine.

In immunoblots, antibody binding to the class 2 and 3 proteins is dependent upon renaturation in a dipolar-ionic detergent (23). The postvaccination antibodies bound much better to the class 2 protein when the IgG response to the serotype 2a protein was examined in the presence of the detergent Empigen BB (37) than when the detergent was absent. However, the binding level was quite variable among individuals. The response to the class 1 protein remained highest.

Recent Clinical Studies

A number of clinical studies have been conducted with OMP vaccines; all of them indicate that these vaccines are safe and immunogenic. Most individuals will experience local reactions of redness or tenderness at the injection site, but systemic reactions are minimal (11).

Rosenqvist et al. (29) examined the antibody responses of 57 Norwegian adults to a vaccine containing OMPs of serotypes 2b:P1.2 and 15:P1.16 noncovalently complexed to meningococcal PSs A, C, Y, and W135. Volunteers received one dose of the vaccine containing 52 μg of protein from each serotype, 40 μg of each PS, and 3.5 μg of lipopolysaccharide. Overall, 70% of the vaccinees had immune responses to both serotypes as judged by the enzyme-linked immunosorbent assay and bactericidal assay. The vaccinee responses were equivalent to those of group B meningococcal disease patients, who had mostly B:15:P1.16. The antibody response was predominantly IgG, as we had found in similar studies (11). The majority of the vaccinees had substantial increases in bactericidal antibodies to both serotypes included in the vaccine. Before vaccination, 60% of the individuals had no detectable bactericidal activity against either serotype, and after immunization, only 21 and 24% lacked bactericidal antibodies against types 2b and 15, respectively. A second immunization or use of adjuvants would probably improve the response.

An important observation of Rosenqvist et al. (29) was the excellent correlation ($r = 0.8$) between the IgG levels measured by the enzyme-linked immunosorbent assay and bactericidal titers measured by using human complement. Earlier studies in which baby rabbit serum was used as a source of complement failed to show a correlation between the two assays.

The major target population for a group B vaccine is young children. We have therefore compared the immune responses of adults and children to vaccines consisting of serotype 2a OMP noncovalently complexed with group B meningococcal PS (9). The antibody responses were measured by the enzyme-linked immunosorbent assay with purified serotype 2a outer membrane vesicles and by a bactericidal assay with a group C:2a strain and baby rabbit serum complement. In both assays young children (under 6 years old) had lower antibody responses than did older

children and adults. Over 50% of children over 2 years of age had postimmunization bactericidal titers of 1:8 or greater, compared with only 20% for those under 2 years of age. By comparison, 70 to 80% of adults had a fourfold rise in their bactericidal titer after immunization (8).

In an effort to improve the percentage of young children developing bactericidal antibodies following administration of serotype vaccines, we have evaluated the effect of adsorption of serotype 2b protein vaccines onto aluminum hydroxide. Initial studies in animals indicated that adsorption of the vaccine significantly improved the immune response (9). The experiments have now been extended to studies with adult volunteers (11). Groups of adults were immunized with a serotype 2b-group B PS vaccine with and without aluminum hydroxide adjuvant. The adjuvant improved the antibody response as measured by the enzyme-linked immunosorbent assay and bactericidal assay. Only those who received the first immunization with adjuvant had a booster effect on reimmunization. These studies indicate that aluminum hydroxide or other adjuvants should be used to increase the percentage of children who respond to OMP vaccines.

Efficacy Trials

Trials to estimate the efficacy of OMP vaccines for preventing group B meningococcal disease have been conducted in South Africa and are now in progress in Cuba and Chile. A randomized serotype 2a vaccine trial was carried out in Cape Town, South Africa, in 1981 (10). The vaccine was administered to 2,200 children between 4 months and 6 years of age (mean, 2 years). Another 2,200 received a group A and C PS vaccine. The trial was conducted during a group B serotype 2b epidemic and provided evidence for protection against group B serotype 2 disease, but insufficient numbers of children were immunized to allow for a statistical estimate of efficacy. Results are not yet available for the other trials.

Problems with Serotype Protein Vaccines

One problem with the serotype protein approach to prevention of group B meningococcal disease is the restricted range of protection induced by the serotype and subtype proteins. To achieve broad protection, a vaccine would have to contain OMPs from at least three or four serotypes, and inclusion of this number in a vaccine is feasible.

The serotypes associated with significant levels of disease vary from one geographic region to another, as well as over a period of years. Continued epidemiological surveillance of disease-associated serotypes should be done to produce the optimal vaccine for a given period and location.

Although serotype protein vaccines offer the best immediate approach, there may be alternatives. An ideal meningococcal vaccine would be immunogenic in all age groups and protect against all group B strains and possibly all meningococci. However, such a vaccine can be developed only when there is a much better understanding of the basic mechanisms by which the organism is able to gain entrance into the host, evade host defenses, and cause invasive disease. Alternative antigens that must be examined include the high-molecular-weight, iron-inducible OMPs found in all meningococci. These and other surface proteins, such as stress proteins, are induced or greatly increased under in vivo growth conditions. We know, for instance, that patients respond strongly to some high-molecular-weight OMPs that appear only as very minor proteins when the organisms are grown on the usual laboratory media. Therefore, membrane vaccines should be prepared from organisms induced to elaborate these proteins. Other alternative antigens include fimbriae and lipopolysaccharide-derived oligosaccharides covalently bound to a protein carrier.

In conclusion, the existing meningococcal PS vaccines are safe and effective and should be used to control outbreaks due to serogroups A, C, Y, and W135. In severe group B outbreaks, in which only one or two serotypes are responsible for most cases of the disease, a serotype protein vaccine is likely to be valuable. I am confident that serotype protein vaccines will protect against group B and group C disease, but I am concerned that such protection may not provide sufficient coverage; that is, protection may be too serotype restricted. For broad protection, the vaccine potential of alternative cell surface components must be evaluated. When an effective group B meningococcal vaccine is developed, this vaccine would ideally be combined with the other meningococcal PSs, the *H. influenzae* type b PS, and possibly some of the pneumococcal PSs, because our ultimate goal is to prevent the most common causes of pediatric bacterial meningitis.

LITERATURE CITED

1. **Artenstein, M. S., R. Gold, J. G. Zimmerly, F. A. Wyle, H. Schneider, and C. Harkins.** 1970. Prevention of meningococcal disease by group C polysaccharide vaccine. N. Engl. J. Med. **282**:417–420.
2. **Beuvery, E. C., A. Kaaden, V. Kanhai, and A. B. Leussink.** 1983. Physiochemical and immunological characterization of meningococcal group A polysaccharide-tetanus toxoid conjugates prepared by two methods. Vaccine **1**:31–36.
3. **Beuvery, E. C., F. Miedema, R. Van Delft, and K. Haverkamp.** 1983. Preparation and immunochemical characterization of meningococcal group C polysaccharide-tetanus toxoid conjugates as a new generation of vaccines. Infect. Immun. **40**:39–45.
4. **Brodeur, B. R., Y. LaRose, P. Tsang, J. Hamel, F. A. Ashton, and A. Ryan.** 1985. Protection against infection with *Neisseria meningitidis* group B serotype 2b by passive immunization with serotype-specific monoclonal antibody. Infect. Immun. **50**:510–516.
5. **Centers for Disease Control.** 1985. Recommendations of the Immunization Practices Advisory Committee (ACIP)—meningococcal vaccines. Morbid. Mortal. Weekly Rep. **34**:255–259.
6. **Densen, P., J. M. Weiler, J. M. Griffiss, and L. G. Hoffmann.** 1987. Familial properdin deficiency and fatal meningococcemia: correction of the bactericidal defect by vaccination. N. Engl. J. Med. **316**:922–926.
7. **Eskola, J., H. Peltola, A. K. Takala, H. Kayhty, M. Hakulinen, V. Karanko, E. Kela, P. Rekola, P.-R. Ronnberg, J. S. Samuelson, L. K. Gordon, and P. H. Makela.** 1987. Efficacy of Haemophilus influenzae type b polysaccharide-diphtheria toxoid conjugate vaccine in infancy. N. Engl. J. Med. **317**:717–722.
8. **Frasch, C. E.** 1983. Immunization against Neisseria meningitidis, p. 115–144. *In* C. S. F. Easmon and J. Jeljaszewicz (ed.), Medical microbiology, vol. 2. Academic Press, Inc., New York.
9. **Frasch, C. E., G. J. Coetzee, L. Wu, L. Y. Wang, and E. Rosenqvist.** 1987. Immune response of adults and children to group B Neisseria meningitidis outer membrane protein vaccines, p. 262–272. *In* J. B. Robbins (ed.), Bacterial vaccines. Praeger Publications, New York.
10. **Frasch, C. E., G. Coetzee, J. M. Zahradnik, H. A. Feldman, and H. J. Koornhof.** 1983. Development and evaluation of group B serotype 2 protein vaccines: report of a group B field trial. Med. Trop. **43**:177–183.
11. **Frasch, C. E., J. M. Zahradnik, L. Y. Wang, L. F. Mocca, and C.-M. Tsai.** 1988. Antibody response to adults to an aluminum hydroxide adsorbed Neisseria meningitidis serotype 2b protein group B-polysaccharide vaccine. J. Infect. Dis. **158**:710–718.
12. **Frasch, C. E., W. D. Zollinger, and J. T. Poolman.** 1985. Serotype antigens of Neisseria meningitidis and a proposed

scheme for designation of serotypes. Rev. Infect. Dis. **7**:504–510.

13. **Gold, R.** 1979. Immunogenicity of meningococcal polysaccharides in man, p. 121–151. *In* J. Rudbach and P. Baker (ed.), Immunology of bacterial polysaccharides. Elsevier Science Publishing, Inc., New York.
14. **Gotschlich, E. C., I. Goldschneider, and M. S. Artenstein.** 1969. Human immunity to the meningococcus IV. Immunogenicity of group A and group C polysaccharides in human volunteers. J. Exp. Med. **129**:1367–1384.
15. **Gotschlich, E. C., M. Rey, R. Triau, and K. J. Sparks.** 1972. Quantitative determination of the human immune response to immunization with meningococcal vaccines. J. Clin. Invest. **51**:89–96.
16. **Granoff, D. M., and R. S. Munson, Jr.** 1986. Prospects for prevention of Haemophilus influenzae type b disease by immunization. J. Infect. Dis. **153**:448–461.
17. **Jennings, H. J., A. Gamian, and F. E. Ashton.** 1987. N-Propionylated group B meningococcal polysaccharide mimics a unique epitope on group B Neisseria meningitidis. J. Exp. Med. **165**:1207–1211.
18. **Jennings, H. J., and C. Lugowski.** 1981. Immunochemistry of groups A, B, and C meningococcal polysaccharide-tetanus toxoid conjugates. J. Immunol. **127**:1011–1018.
19. **Jennings, H. J., R. Roy, and A. Gamian.** 1986. Induction of meningococcal group B polysaccharide-specific IgG antibodies in mice using an N-propionylated B polysaccharide-tetanus toxoid conjugate vaccine. J. Immunol. **137**:1708–1713.
20. **Kabat, E. A., H. Kaiser, and H. Sikorski.** 1945. Preparation of the type-specific polysaccharide of the type I meningococcus and a study of its effectiveness as an antigen in human beings. J. Exp. Med. **80**:299–307.
21. **Kayhty, H., V. Karanko, H. Peltola, S. Sarna, and P. H. Makela.** 1980. Serum antibodies to capsular polysaccharide vaccine of group A Neisseria meningitidis followed for three years in infants and children. J. Infect. Dis. **142**:861–868.
22. **Lepow, M. L., J. Beeler, M. Randolph, J. S. Samuelson, and W. A. Hankins.** 1986. Reactogenicity and immunogenicity of a quadravalent combined meningococcal polysaccharide vaccine in children. J. Infect. Dis. **154**:1033–1036.
23. **Mandrell, R. E., and W. D. Zollinger.** 1984. Use of a zwitterionic detergent for the restoration of the antibody-binding capacity of electroblotted meningococcal outer membrane proteins. J. Immunol. Methods **67**:1–11.
24. **Moreno, C., M. R. Lifely, and J. Esdaile.** 1985. Immunity and protection of mice against *Neisseria meningitidis* group B by vaccination, using polysaccharide complexed with outer membrane proteins: a comparison with purified B polysaccharide. Infect. Immun. **47**:527–533.
25. **Moreno, C., M. R. Lifely, and J. Esdaile.** 1985. Effect of aluminum ions on chemical and immunological properties of meningococcal group B polysaccharide. Infect. Immun. **49**: 587–592.
26. **Peltola, H., P. H. Makela, H. Kayhty, H. Jousimies, E. Herva, K. Hallstrom, A. Sivonen, O.-V. Renkonen, O. Petty, V. Karanko, P. Ahonen, and S. Sarna.** 1977. Clinical efficacy of meningococcus group A capsular polysaccharide vaccine in children three months to five years of age. N. Engl. J. Med. **297**:686–691.
27. **Peltola, H., A. Safary, H. Kayhty, V. Karanko, and F. E. Andre.** 1985. Evaluation of 2 tetravalent (ACYW135) meningococcal vaccines in infants and small children—a clinical study comparing immunogenicity of O-acetyl-negative and O-acetyl-positive group C polysaccharides. Pediatrics **76**:91–96.
28. **Reingold, A. L., C. V. Broome, A. W. Hightower, G. W. Ajello, G. A. Bolan, C. Adamsbaum, E. E. Jones, C. Phillips, H. Tiendrebeogo, and A. Yada.** 1985. Age-specific differences in duration of clinical protection after vaccination with polysaccharide A vaccine. Lancet **ii**:114–118.
29. **Rosenqvist, E., S. Harthug, L. O. Froholm, E. A. Hoiby, K. Bovre, and W. D. Zollinger.** 1988. Antibody responses to serogroup B meningococcal outer membrane antigens after vaccination and infection. J. Clin. Microbiol. **26**:1543–1548.
30. **Ross, S. C., and P. Densen.** 1984. Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. Medicine **63**:243–273.
31. **Sanborn, W. A.** 1987. Development of meningococcal vaccines, p. 121–134. *In* N. A. Vedros (ed.), Evolution of meningococcal disease, vol 2. CRC Press, Inc., Boca Raton, Fla.
32. **Saukkonen, K., H. Abdillahi, J. T. Poolman, and M. Leinonen.** 1987. Protective efficacy of monoclonal antibodies to class 1 and class 3 outer membrane proteins of Neisseria meningitidis B:15:P1.16 in infant rat infection model: new prospects for vaccine development. Microb. Pathogen. **3**:261–267.
33. **Sjoholm, A. G., E. J. Kuijper, C. C. Tijssen, A. Jansz, P. Bol, L. Spanjaard, and H. C. Zanen.** 1988. Dysfunctional properdin in a Dutch family with meningococcal disease. N. Engl. J. Med. **319**:33–37.
34. **Tsai, C.-M., C. E. Frasch, and L. F. Mocca.** 1981. Five structural classes of major outer membrane proteins in *Neisseria meningitidis*. J. Bacteriol. **146**:69–78.
35. **Vedros, N. A.** 1987. Development of meningococcal serogroups, p. 33–38. *In* N. A. Vedros (ed.), Evolution of meningococcal disease, vol 2. CRC Press, Inc. Boca Raton, Fla.
36. **Wedege, E., and L. O. Froholm.** 1986. Human antibody response to a group B serotype 2a meningococcal vaccine determined by immunoblotting. Infect. Immun. **51**:571–578.
37. **Wedege, E., and T. E. Michaelsen.** 1987. Human immunoglobulin G subclass immune response to outer membrane antigens in meningococcal group B vaccine. J. Clin. Microbiol. **25**:1349–1353.
38. **Whadan, M. H., F. Rizk, A. M. El-Akkad, A. A. El Ghoroury, R. Hablas, N. I. Girgis, A. Amer, W. Boctar, J. E. Sippel, E. C. Gotschlich, and R. Triau.** 1973. A controlled field trial of serogroup A meningococcal polysaccharide vaccine. Bull. W.H.O. **48**:667–673.
39. **Williamson, W. A., and B. M. Greenwood.** 1978. Impairment of the immune response to vaccination after acute malaria. Lancet **i**:1328–1329.
40. **Zollinger, W. D., R. E. Mandrell, J. M. Griffiss, P. Altieri, and S. Berman.** 1979. Complex of meningococcal group B polysaccharide and type 2 outer membrane protein immunogenic in man. J. Clin. Invest. **63**:836–848.