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POST-SYMPOSIUM LECTURE

PERSPECTIVES ON PRODUCTION OF GROUP B MENINGOCOCCAL VACCINES

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ABSTRACT: Group B meningococcal disease remains a problem in many countries. Since the group B *Neisseria meningitidis* polysaccharide has proven not to induce protective antibodies, existing vaccine against group B disease have been composed of lipopolysaccharide depleted outer membranes, usually in the form of small vesicles. Protection against meningococcal disease is correlated with induction of bactericidal antibodies. The group B vaccines stimulating the highest bactericidal titers when administered in a two dose immunization series 6 to 8 weeks appart consist of soluble vesicles, and one of the meningococcal polysaccharides all adsorbed to aluminum hydroxide. Efficacy trials with such vaccines have recently been conducted in Chile, Cuba, and Norway. The Cuban trial demonstrated 80% efficacy against disease caused by a B:4:P1.15 strain, and was the first to clearly demonstrate that antibodies induced to non-capsular antigens can protect against meningococcal disease.
KEY WORDS: *Neisseria meningitidis*, vaccine, outer membrane

INTRODUCTION

Serogroup B *Neisseria meningitidis* is responsible for over 80% of meningococcal diseases in Brazil and is the predominant cause of meningococcal disease in many oth-

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er countries including the United States. A recent study of serogroup prevalence in the United States[26] showed that approximately equivalent amounts of meningococcal disease were due to groups B and C. However, the incidence of meningococcal disease remains approximately 1/100,000 in the US compared to 2.5/100,000 in Brazil.

GROUP B POLYSACCHARIDE

Effective capsular polysaccharide vaccines against *N. meningitidis* serogroups A and C were developed in the early 1970s[18]. Later the Y and W135 polysaccharides were added to produce a tetravalent meningococcal vaccine[2]. Although the group B polysaccharide would be the logical choice for a group B vaccine, it is poorly immunogenic and antibodies induced by the polysaccharide do not appear to be protective. The B polysaccharide is a homopolymer of alpha 2-8 linked N-acetyl neuraminic acid, the same as on some fetal proteins. This may account for the observed poor immunogenicity of the B polysaccharide.

Attempts have been made to improve the immunogenicity of the polysaccharide [22,27,28]. Adsorption of the polysaccharide to aluminum hydroxide appeared promising in mice[28], but failed to increase immunogenicity in humans[14]. Another approach was to prepare a chemically modified polysaccharide, in which the N-acetyl groups on the polysaccharide were replaced with N-propionyl groups[21,22]. When this altered polysaccharide was chemically bound to tetanus toxoid, forming a conjugate vaccine, the N-propionyl polysaccharide induced bactericidal antibodies. Conjugates prepared using the native group B polysaccharide were nonimmunogenic. Studies are continuing by Dr. Jennings to evaluate this approach.

MENINGOCOCCAL OUTER MEMBRANE ANTIGENS

Protection against meningococcal disease is correlated with the presence of bactericidal antibodies[18]. 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 a 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. Thus, a group B meningococcal vaccine should be based on cell surface antigens shown to induce bactericidal antibodies, although opsonic antibodies probably also contribute to protection. Studies have shown that convalescent sera from patients with group B meningococcal disease have bactericidal antibodies directed against surface proteins and the lipopolysaccharide[19,23,32]. Most efforts to develop an effective group B vaccine have, therefore, involved use of outer membrane proteins[8,41].

There is a large amount of antigenic diversity among group B meningococcal strains. The outer membrane contains three to five major proteins, comprising 5 protein classes, class 1 through class 5 having molecular weights between 26,000 and 46,000 daltons[36]. There are approximately 20 different serotypes within group B and group C based upon immunologic differences in the Class 2 and Class 3 major OMPs which are between 34K and 40K[15]. These clas 2/3 proteins are the meningococcal porins. Further antigenic diversity is seen among the approximately 46K Class 1 OMPs, which contain

the subtype specific antigens and may also have porin function. Antibodies to both the serotype and subtype proteins are bactericidal.

Although sporadic causes of group B meningococcal disease may be caused by a variety of serotypes, outbreaks and epidemics in most countries have been caused by a small number of serotypes, 2, 4, 8 and 15[1,10]. Thus, a serotype protein vaccine need contain membranes from a relatively small number of serotypes. In addition to our laboratory, laboratories in Biltoven, The Netherlands[30]; Havana, Cuba[34]; Oslo, Norway [16] and Washington DC, USA[41] have produced group B meningococcal vaccines, all based on use of lipopolysaccharide depleted outer membranes.

EARLY OUTER MEMBRANE VACCINES

Other membranes may be removed from meningococci by lithium chloride-sodium acetate extraction at 50°C. These membranes contain approximately equivalent amounts of protein and toxic lipopolysaccharide (LPS). For vaccine use, the LPS must be largely eliminated by selective solubilization with detergents, a number of which have been used for this purpose including Brij-96[13], Empigen-BB[40], and sodium deoxycholate[37]. Sodium deoxycholate may be best because it is a normal bile metabolite present in humans, and residual detergent that may be present in the vaccine would probably not have toxic effects.

To prepare OMV vaccines free of unknown quantities of group B meningococcal polysaccharide, we have isolated non-encapsulated variants or mutants using horse anti-group B polysaccharide serum incorporated into a clear agar medium to detect colonies not elaborating the B polysaccharide[13].

The first outer membrane vaccines consisted of LPS depleted membranes. The vaccine protein was separated from the detergent by ethanol precipitation and resuspended in 0.9% sodium chloride.

These vaccines were visibly particulate and contained aggregated outer membranes as observed by electron microscopy. Later studies showed that the membranes were soluble in water, but not 0.9% sodium chloride.

The early particulate vaccines as well as later vaccines contained considerable amounts of LPS (about 5 to 10 μg/100 μg protein), yet were much less pyrogenic in rabbits than would be expected[35]. The LPS that remained was strongly membrane associated, which probably accounted for the lower toxicity.

The particulate outer membrane vaccines were evaluated in adults then in children using a two or three dose immunization schedule previously evaluated in animal studies[8]. Zollinger et al.[42] found that such a vaccine failed to induce bactericidal antibodies in five adults after three doses. A similar particulate vaccine prepared in our laboratory induced low levels of antibody in both adults and children as measured by ELISA, but also failed to stimulate bactericidal antibodies. Thus, although particulate vaccines were found safe in both adults and children, they were poorly immunogenic, a fact not predicted by the animal studies.

Zollinger et al. were first to clinically evaluate soluble outer membrane vaccines[42]. They found that outer membrane vaccines could be made soluble by combination with group B meningococcal polysaccharide. Electron microscopy of similar vaccines prepared in our laboratory showed some aggregation of outer membrane vesicles (OMV) without the polysaccharide and individual vesicles with polysaccharide[13].

Meningococci release large amounts of essentially pure outer membranes during normal growth into the culture broth as blebs or vesicles[7,13]. These membranes may be purified from the broth and used as the starting material for preparation of a vaccine[13]. Since the natural orientation of the proteins in the outer membrane may be important, we have developed methods to selectively remove the LPS, leaving the membranes intact and soluble as determined by electron microscopy[13]. Soluble vaccines were prepared with and without the group B polysaccharide and tested in animals[29,37].

The soluble OMV vaccines are colloidal suspensions rather than true solutions. Increasing the ionic strength caused precipitation of the OMV, whereas addition of a negatively charged polysaccharide increased OMV solubility. The polysaccharide forms a non-covalent complex with the vesicles[43]. Recent results from our laboratory show that the OMV-polysaccharide association is hydrophobic, because removal of the lipid tail from the polysacharide by phospholipase blocked the interaction.

Soluble protein plus polysaccharide vaccines have been clinically evaluated[8,43]. These vaccines induced bactericidal antibodies on primary immunization with only modest increases in antibody titers after the second immunization. We then compared the immunogenicity of a soluble OMV vaccine with and without group B polysaccharide[8,33]. Addition of the polysaccharide resulted in a significant increase in bactericidal antibodies to a group C serotype 2a strain. The second dose 6 to 8 weeks later resulted in an increase in the percent of individuals responding to the vaccine.

The target age group for a group B vaccine is young children. When the immune responses of children were compared to those of older children and adults, by either OMV ELISA or bactericidal assay, young children (under 6 years old) responded less well[12]. In an effort to increase the percentage of young children developing bactericidal antibodies, the OMV vaccine was adsorbed onto aluminum hydroxide or[14].

Adsorption of the OMV plus polysaccharide onto aluminum hydroxide or aluminum phosphate significantly increased the bactericidal response of mice to the outer membrane proteins[37]. These vaccines were therefore evaluated in human adults[14]. The aluminum hydroxide absorbed vaccine was found safe and more immunogenic than the same vaccine without the adjuvant. The vaccine in combination with the adjuvant induced significantly higher bactericidal titers. More recent studies in Norway suggest that the adjuvant can be added directly to LPS depleted membranes[20].

In summary, these data provide evidence indicating that to stimulate an optimal immune response, the surface exposed protein epitopes need to be presented to the immune system in a near-native configuration, and therefore should remain within soluble membranes.

CURRENT VACCINES AND RECENT CLINICAL STUDIES

A study in Norway[32] using a combined serotype 2b and serotype 15 OMV vaccine showed that bactericidal antibodies were induced to both serotypes in 70% of the adults tested. This demonstrated the utility of combining multiple serotypes. The study also showed a good correlation between IgG antibodies to the outer membranes and bactericidal titers measured using human complement.

For comparative clinical studies in Norway[20] three different vaccine formulations were prepared containing OMV from a B:15:P1.16 strain mixed with either group C meningococcal polysaccharide, or with aluminum hydroxide, or with both. These vaccine

formulations were evaluated in adults and students in preparation for a large scale efficacy trial. Two doses of protein between 12.5 and 100 μg per dose were given at a 6 week interval. The researchers found equivalent bactericidal responses when the proteins were administered adsorbed to aluminum hydroxide with or without the group C polyssachariде, and that the 50 μg dose was optimal.

A group B vaccine has been produced in Cuba consisting of LPS depleted OMV from a B:4:P1.15 strain, a high molecular weight protein complex, and group C meningococcal polysaccharide, all adsorbed onto aluminum hydroxide[34]. Care is taken to control the vesicle structure and size. The vaccine contains per dose 50 μg protein, 50 μg polysaccharide, and 2 mg aluminum hydroxide. It is administered as a two dose immunization schedule with a 6 to 8 week interval. The dosage and interval were arrived at after evaluation of different immunization schedules in adults and children. Eighty-eight percent of school children responded with 2-fold or greater increases in outer membrane antibodies as measured by ELISA.

GROUP B EFFICACY TRIALS

A number of efficacy trials have now been conducted with varying results using group B meningococcal vaccines (Table 1). A number of important observations can be drawn from these trials, but the foremost is that antibodies to non-capsular surface antigens can prevent group B meningococcal disease[34]. The first trial was carried out in Cape Town, South Africa in 1981[9] against a B:2b:P1.2 epidemic (peak incidence; 150/100,000) using a 2a:P1.2 outer membrane vaccine combined with group B polysaccharide, but no adjuvant. Although insufficient cases occurred to estimate efficacy, no serotype 2 disease occurred in the vaccinated children, but equivalent amounts of disease due to other group B serotypes occurred in vaccinated and control children (received meningococcal AC vaccines). Thus the vaccine failed to protect against nonserotype 2 disease.

An efficacy trial was performed in Iquique, Chile in 1988-1990 using an outer membrane protein vaccine from a B:15:P1.3 strain combined with group C meningococcal

TABLE I

Field trials of group B meningococcal outer membrane vaccines

Years	Vaccine formulation	Location	Est. Efficacy	Ref.
1981-82	2a:P1.2 + B polysacch	Cape Town, South Africa	Too few cases	9
1987-89	4:P1.15 + C polysacch + Al(OH)3	Cuba	80%	34
1988-90	15:P1.3 + C polysacch + Al(OH)3	Iquique, Chile	51%	5
1989-91	15:P1.16 +Al(OH)3	Norway	In progress	16

polysaccharide and aluminum hydroxide[5]. During the trial over 90% of the group B meningococcal disease was due to the B:15:P1.3 clone. In this double-blind trial 40,800 volunteers, ages 1-21 years, received two doses of either the B vaccine or ACYW135 polysaccharide vaccine given 6 weeks appart. The estimated efficacy was 51%. The vaccine differed from other vaccines in that efforts were taken to reduce the LPS content to very low levels, which probably disrupted the membrane structure. The antibody responses of the children as measured by ELISA were good, but the numbers responding with bactericidal titers were low. This illustrates the need for measurement of bactericidal antibodies.

A randomized double-blinded efficacy trial was carried out in Cuba between 1987 and 1989 using the B:4:P1.15 vaccine described above[34]. The trial was conducted in 197 boarding schools, randomized by school, where there were 106,000 students between 10 and 14 years of age, half of which received the serotype 4 vaccine. During the trial 95% of group B disease was due to B:4:P1.15 and 3% to B:15:P1.15. Thus, this vaccine, like the vaccine used in Chile, was evaluated against a single group B clone. The estimated efficacy was 80%, clearly demonstrating that a protein vaccine can prevent B:4:P1.15 disease. However, since the epidemic was caused by one clone, the trial was not able to provide evidence for the degree of protection that could be expected against other group B strains.

IMPORTANT VACCINE CHARACTERISTICS AND PROBLEMS WITH CURRENT VACCINES

Immunogenicity and efficacy studies conducted with a different outer membrane vaccines have demonstrated a number of physical characteristics that are important for optimal immunogenicity of these vaccines (Table 2). A number of studies have shown that isolated outer membrane proteins induce few antibodies reactive against surface exposed epitopes. These proteins have loop structures crossing the membrane several times[25], that are not conserved upon removal from a membrane environment. We have therefore sought to maintain the vesicle structure, following detergent treatment to remove the LPS, and this is monitored by electron microscopy. Additional reasons to work with the intact membranes are that antibodies to no one protein are likely to pro-

TABLE II

Important characteristics of a meningococcal outer membrane protein vaccine

1. Must be soluble – Solubility improved by addition of polysaccharide
2. Native conformation of proteins should be maintained by: Retaining outer membrane structure OR Insertion into liposomes
3. Maintain approximately 50 μg of LPS per mg protein to retain near native outer membrane conformation
4. Outer membrane proteins normally expressed during infection should be included: Iron regulated proteins Stress proteins (heat shock)

vide broad protection against group B meningococcal disease, and we do not yet know to which proteins the critical protective antibodies are directed.

A minimum amount of LPS is required to maintain the outer membrane conformation, without which the membranes disintegrate. Isolated outer membranes contain approximately equal quantities of protein and LPS. Deoxycholate treatment removes about 95% of the LPS without disrupting the membranes, while stronger detergent treatment to remove additional LPS generally disrupts the membranes.

There are problems with all of the outer membrane protein vaccines that have received clinical evaluation to date. First, the existing vaccines are rather serotype specific. The antigenic composition of the vaccines need to be changed to provide for induction of broadly protective antibodies. In this regard, it now appears that antibodies to no single protein will provide broad protection. Most all of the outer membrane proteins appear to have antigenic variants. Second, the vaccines fail to include a number of important cell surface proteins that are expressed during infection. These in vivo proteins include the iron regulated outer membrane proteins[3,4], and probably heat-shock proteins[39]. Third, the vaccines do not appear to induce a clear booster response as would be expected of a protein antigen when the second immunization is given 6 weeks after the first. In addition, no data have been presented demonstrating whether vaccination primes for a booster response if the child is reimmunized 6 months or a year after the primary two doses immunization series. Most other protein vaccines are given as a multiple immunization series. Lastly, it may be necessary to use genetic engineering to remove the class 4 protein from the vaccine strains. These proteins are equivalent to gonococcal Protein III, and Protein III has been shown to induce blocking antibodies[31]. The class 4 protein has been removed without effecting growth properties [24].

Although vaccines consisting of LPS depleted outer membranes offer the best imediate approach, there are alternatives. An ideal meningococcal vaccine would be immunogenic in all age groups and protect against all group B strains in addition to the other disease associated serogroups, A, C, Y and W135. Development of such a vaccine will likely require a better understanding of the basic mechanisms by which only some meningococcal strains are able to gain enterance into the host and cause disease. Wetzler et al.[38] have successfully used purified outer membrane proteins inserted into liposomal membranes to induce high levels of bactericidal antibodies. Now we only need to know which proteins should be included. Another very promising finding is that alkaline detoxified meningococcal LPS remained immunogenic and induced bactericidal antibodies in mice[6].

RESUMO: A doença meningococcica continua sendo um problema em muitos países. Uma vez que o polissacáride da *Neisseria meningitidis* B mostrou-se incapaz de induzir a formação de anticorpos protetores, as vacinas existentes contra a doença meningocócica pelo grupo B tem apresentado em sua composição, membrana externa com quantidade reduzida de lipopolissacáride sob a forma de vesículas. A proteção contra a doença meningococica está correlacionada com a indução de anticorpos bactericidas. A vacina contra o grupo B estimula a produção de altos títulos de anticorpos bactericidas quando administrada em duas doses com intervalo de 6 a 8 semanas e consiste de vesículas solúveis e um polissacaride de meningococo, todos absorvidos ao hidróxido de alumínio. Testes de eficácia com tais vacinas foram recentemente feitos no Chile, Cuba e Noruega. A vacina Cubana demonstrou 80% de eficácia contra a

doença meningococcica causada por cepas B:4:P1.15, e foi a primeira a demonstrar claramente que anticorpos induzidos por antígenos não capsulares podem proteger contra a doença meningococcica.

PALAVRAS CHAVES: *Neisseria meningitidis*, vacina, membrana externa

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