Antonie van Leeuwenhoek 53: 395–402 (1987)

Appearance of new strains associated with group B meningococcal disease and their use for rapid vaccine development

C. E. FRASCH, L. F. MOCCA & A. B. KARPAS
Office of Biologics, Food and Drug Administration, Bethesda, MD, 20892, USA

Abstract. There has been a decrease in the prevalence of disease in the United States due to meningococcal serotypes 2a and 2b containing class 2 proteins with a concomitant increase in nonserotypable strains containing class 3 major outer membrane proteins. A new disease associated strain was identified using monoclonal antibodies as B:4:P1.15. Serotype 4 strains have been heretofore isolated almost only from carriers. This B:4:P1.15 strain predominated among group B disease isolates in Cuba from the late 1970s to the present and among Miami, Florida isolates recovered in 1981 and 1982. To determine whether protein vaccines for new strains or serotypes could be prepared using our present methods, a combined vaccine was prepared from a group B strain (B:8:P1.15) recovered during a recent outbreak in Virginia, and a serotype 2b strain, plus group C polysaccharide. The vaccine was prepared with aluminum hydroxide, or with trehalose dimycolate plus monophosphoryl lipid A, or without adjuvant. Four weeks after immunization antibody levels were much higher in mice that received vaccine containing adjuvant.

Introduction

Group B is the predominant cause of meningococcal disease in the United States as well as many European countries, and is the only important meningococcal serogroup for which there is no effective polysaccharide vaccine. Serogroup B has been subdivided into over 15 different serotypes based upon the immunological specificity of the class 2 or class 3 major outer membrane proteins (Frasch et al. 1985b), but only a few of these serotypes have been associated with outbreaks of meningococcal disease, and their prevalence changes with time. Other serotypes such as types 4, 5, and 6 have been isolated almost exclusively from carriers (Frasch & Chapman 1973). Subtypes within the serotypes have been defined based upon the antigenic specificity of the class 1 proteins as in B:15:P1.15 (group: serotype: subtype, Frasch et al. 1985b).

Outer membrane protein vaccines have been clinically evaluated and induce bactericidal antibodies, which are strongly associated with protection against meningococcal disease (Frasch et al. 1985a; Zollinger et al. 1979). Antibodies against some cell surface proteins, including the class 1 and class 2 proteins, are bactericidal (Zollinger & Mandrell 1983). The same class 1 serosubtype protein may be shared to some extent among different serotypes (Zollinger et al.

1984), and are included in group B protein vaccines to extend protection.

The present study presents a preliminary analysis of a newly characterized virulent group B serotype 4 strain[1]. An outer membrane protein vaccine was prepared using existing methods from a recent group B outbreak strain and evaluated in mice.

Materials and methods

The serotype antigens were salt-extracted and subjected to Ouchterlony serotyping as previously described (Craven et al. 1979). Strains were also serotyped using monoclonal antibodies in a dot-immunoblot procedure (Hawkes et al. 1982).

Vaccines were prepared containing 50 ug/ml of LPS-depleted outer membrane vesicles (OMV) from strains 3006 (B:2b:P1.2) and BB-431 (B:8:P1.5) noncovalently complexed with 50 ug/ml of purified group C polysaccharide. Strain BB-431 was recovered during a group B meningococcal outbreak in Staunton, Virginia in 1986. Purified OMVs extracted as described for serotype antigen preparation (Mocca et al. 1982) were dissolved in 1% sodium deoxycholate in 0.1 M Tris-HCl, 0.002 M EDTA, pH 8.5, layered onto a 60% sucrose bed, and centrifuged at 100,000 × G for 4 h. The LPS-depleted OMVs pelleted onto the sucrose bed, were recovered and redissolved in the detergent solution, then sterile filtered. The protein was precipated and washed twice with filtered 95% ethanol, and used to prepare vaccines with and without adjuvants. An aluminum hydroxide adsorbed vaccine was prepared by mixing equal protions of bulk vaccine and 4 mg/ml sterile aluminum hydroxide. A RAS (Ribi Immumochem, Hamilton, Montana) adjuvanted vaccine was prepared by injection of the saline vaccine into a vial containing RAS (trehalose dimycolate plus monophosphoryl lipid A) as directed by the manufacturer.

NIH general purpose outbred mice weighing 12 to 15 grams were immunized intraperitoneally with 1.0 or 0.1 ug of each protein and an equivalent amount of group C polysaccharide in saline, aluminum hydroxide or RAS. Groups of animals were bled 2 and 4 weeks after a single immunization. Antibody responses were measured by ELISA (Peppler & Frasch 1982) using purified OMV in 96-well polystrene plates (Immunolon I, Dynatech).

[1] This serotype was reported at the Fifth International Pathogenic *Neisseriae* Conference (Noordwijkerhout, The Netherlands) to be a new serotype, type 22, but further analysis of the monoclonal typing reagent indicated that it was actually serotype 4.

Table 1. Occurance of a virulent group B serotype 4 strain (B:4:P1.15) among patient and carrier isolates.

Geographic area	Year	Source	n =	Serotype*			
				4:P1.15	4:N	N:P1.15	Other
Cuba	1979–1980	Disease	7	6	0	0	1
	1983–1986	Disease	9	6	0	1	2
Miami	1981	Disease	10	4	1	0	5
Florida		Carrier	5	1	0	0	4
	1982	Disease	8	7	1	0	0
		Carrier	2	1	0	0	1
Dominican Republic	1984	Disease	3	0	0	0	3
Brazil	1986	Disease	5	2	1	0	2
California	1986	Disease	12	2	3	1	6

* OVMs from strains were tested by dot-blotting using the serotype 4 monoclonal antibody 2303-C5 and P1.15 antibody 2731-C6. N means negative with the respective monoclonal.

Results

Identification of a new virulent strain

Group B meningococcal outbreaks occurred in Miami, Florida in 1981 and 1982 (Caugent et al. 1986). The responsible strain cross-reacted with rabbit antisera to the serotype 15 strain H355 (B:15:P1.15). Representative SDS-PAGE patterns of OMV from the Miami strains are shown in Fig. 1. The strain had a class 3 protein of higher molecular weight than serotype 15, but had the same molecular weight class 1 (P1) protein as H355 (type 15 prototypes), and this accounted for the serotype 15 cross-reactivity.

A representative Miami strain (BB-368) was used to prepare monoclonal antibody (2303-C5), which by dot-blot analysis reacted with all of the epidemiologically related strains and with those having the same protein pattern on SDS-PAGE. The antibody reacted with the class 3 protein by immunoblot (Western) analysis. This monoclonal antibody was tested against OMV from all described serotypes having a class 3 protein, and reacted only with the serotype 4 OMV. Comparison of the OMV from BB-368 and M981 (serotype 4) showed that they had class 3 proteins of the same molecular weight. Based on this analysis strain B-368 was serotype 4.

Table 1 shows preliminary information regarding the geographic distribution of strains having the serotype 4 and P1.15 proteins. Analysis of strains obtained from Cuba prior to the first outbreak in Miami indicated that this strain was

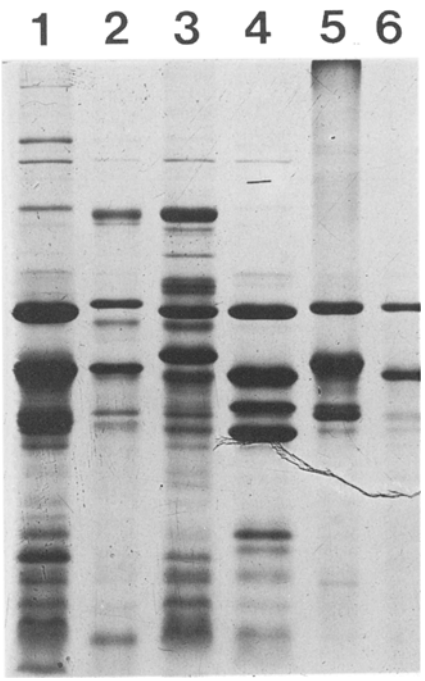

Fig. 1. SDS-polyacrylamide gel analysis of the outer membrane proteins from the Miami (BB-368) and Staunton, Virginia (BB-431) strains. OMVs of the following strains were compared: *(lane 1)* H355 (B:15:P1.15); *(lane 2)* 44/76 (B:15:P1.16); *(lane 3)* BB-368 (B:4:P1.15); *(lane 4)* BB-431 (B:8:P1.15); *(lane 5)* M1080 (B:1); *(lane 6)* M978 (B:8).

prevalent there prior to 1981. This strain is still a significant cause of group B meningococcal disease in a number of countries.

A new meningococcal protein vaccine

An outer membrane protein preparation was prepared from a 1986 group B Virginia outbreak strain (BB-431) for combination in a vaccine with serotype 2b. The protein composition of the LPS depleted OMV from the two serotypes and the combined vaccine is shown in Fig. 2.

The antibody response of the mice to the serotype 2b and BB-431 OMVs was measured by ELISA two and four weeks after immunization. A marked difference in the antibody response to the two vaccine components was found, but this difference is probably due to the differences in the type 2b and BB-431 protein concentrations in the final vaccine (see Fig. 2). Taking this into account, the antibody responses to the 0.1 ug dose of type 2b and the deficient 1 ug dose of BB-431 were equivalent (data not shown).

The serotype 2b antibody response was measured 2 and 4 weeks after a single

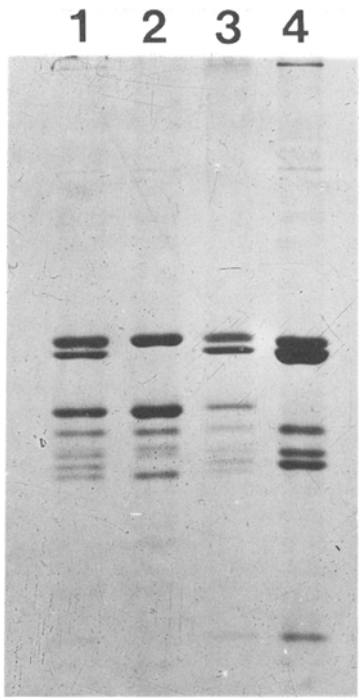

Fig. 2. Analysis of a combined serotype 2b and strain BB-431 outer membrane perotein vaccine and the respective detergent treated OMV components by SDS-PAGE. *(Lane 1)* Material pelleted at 10,000 × g prior to sterile filtration; *(lane 2)* Deoxycholate treated OMV from BB-431 (B:8:P1.15); *(lane 3)* final vaccine (lot 860715VC); *(lane 4)* Deoxycholate treated OMV from 3006 (B:2b:P1.2)

immunization with or without adjuvants (Fig. 3). The antibody response to a 1 ug dose of protein without adjuvant peaked by 2 weeks after immunization. In contrast, the antibody levels were still increasing at four weeks for vaccine given with either adjuvant. The response at 4 weeks with RAS was not significantly different from that obtained with vaccine absorbed to aluminum hydroxide.

Discussion

There are two major divisions within *N. meningitidis,* one containing strains having a class 2 (approx. 41,0000 Mr) and the other with strains having a class 3 (approx. 37,000 Mr) major outer membrane protein (Tsai et al. 1981). No genotypes or electrophoretic types (ET-types) are shared between these two divisions (Caugant et al. 1987).

Serotype 2 strains, which have class 2 major proteins, were responsible for most major outbreaks of group B and group C disease during the 1960s and

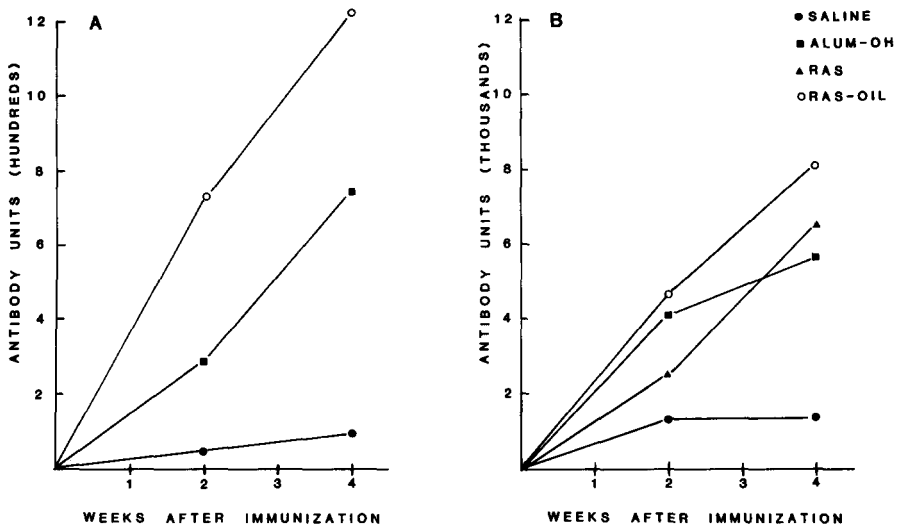

Fig. 3. Serotype 2b antibody response of mice to a single I.P. injection of a combined serotype 2b and strain BB-431 outer membrane protein vaccine with and without adjuvants. There were 8 to 10 mice per group and the geometric mean antibody units/ml are given for *(A)* 0.1 ug dose and *(B)* 1.0 ug dose. Note the difference in scale for A and B.

70s (Frasch & Chapman 1973). Our studies of group B and group C meningococcal strains from a number of recent outbreaks in the USA suggest that a change in the group B strains began in the United States in the 1980s with a shift to strains having class 3 proteins.

We have described a new group B strain of serotype 4, having the subtype P1.15 protein, first described by Froholm et al. (1985). This is of particular interest, because serotype 4 has not been associated with meningococcal disease (Frasch & Chapman 1973). However, the prototype 4 strain M981 does not have the P1.15 subtype protein found on some disease associated serotypes. The strains were identified using monoclonal antibodies, and all strains found to react with the 2303-C5 monoclonal had class 3 outer membrane proteins of the same molecular weight on SDS-PAGE.

Caugant et al. (1986) provided a general description of the Miami and Cuban outbreaks, identifying the causative strain as B:NT:P1.15. They examined these strains for their ET-types by analysis of the electrophoretic mobilities of a series of constitutive metabolic enzymes. They found 7 of 8 Cuban isolates and 14 of 19 Miami disease isolates to be members of the ET-5 complex. By comparison, 6 of 7 Cuban isolates we examined were B:4:P1.15 as were 15 out of 20 Miami disease isolates. Serotype 4 was also found among recent disease isolates from California and Brazil. Additional studies are required to determine the relative importance of the B:4P1.15 strain as a cause of meningococcal disease in different countries.

The strains recovered from the Miami outbreaks were analyzed for sulfonamide sensitivity (Neill, pers. comm.). Of 15 strains identified as B:4:P1.15, 14 were sulfa resistant while only 1 of 16 non-type 4 Miami isolates were sulfa resistant. Thus, sulfa resistance may be another marker for this clone.

A combined BB-431 (B:8:P1.15) and 3006 (B:2b:P1.2) outer membrane protein vaccine was prepared to show that data gathered on the production and immunogenicity of serotype 2 and serotype 15 protein vaccines would be applicable to new strains. Using methods similar to those described previously (Wang & Frasch 1984), we prepared an immunogenic vaccine containing a recent clinical isolate. In agreement with earlier studies (Wang & Frasch 1984) adsorption of the vaccine to aluminum hydroxide significantly improved the antibody responses to both protein components. A clinical study conducted with an aluminum hydroxide adsorbed serotype 2b vaccine (Frasch & Zahradnik 1986) confirmed results obtained using mice (Wang & Frasch 1984). The antibody responses stimulated in the mice by the RAS adjuvanted vaccine at 4 weeks were not significantly different from those obtained with aluminum hydroxide.

References

Caugant, D. A., L. O. Froholm, K. Bovre, E. Holten, C. E. Frasch, L. F. Mocca, W. D. Zollinger & R. K. Selander (1986) Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease. Proc. Natl. Acad. Sci. USA 83: 4927–4931

Caugant, D. A., F. L. Mocca, C. E. Frasch, L. O. Froholm, W. D. Zollinger & R. K. Selander (1987) Genetic structure of *Neisseria meningitidis* populations in relation to serogroup, serotype, and outer membrane protein pattern. J. Bacteriol 169: 2781–2792

Craven, D. E., C. E. Frasch, L. F. Mocca, F. B. Rose & R. Gonzalez (1979) Rapid serogroup identification of *Neisseria meningiditis* by using antiserum agar: prevalence of serotypes in a disease-free military population. J. Clin. Microbiol. 10: 302–307

Frasch, C. E. & S. S. Chapman (1973) Classification of *Neisseria meningitidis* group B into distinct serotypes. III. Application of a new bactericidal inhibition technique to distribution of serotypes among cases and carriers. J. Infect. Dis. 127: 149–154

Frasch, C. E., G. Coetzee, J. M. Zahradnik & L. Y. Wang (1985a) New developments in meningococcal vaccines. In: G. K. Schoolnik (Ed) The Pathogenic *Neisseria* (pp. 633–639). ASM, Washington, D. C.

Frasch, C. E., W. D. Zollinger & J. T. Poolman (1985b) Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. Rev. Infec. Dis. 7: 504–510

Frasch C. E. & J. M. Zahradnik (1986) Human immune response to an aluminum hydroxide absorbed *Neisseria meningiditis* serotype 2b protein vaccine. Antonie van Leeuwenhoek 52: 229–231

Froholm, L. O., E. Holten, J. T. Poolman & W. D. Zollinger (1985) Typing of Norwegian meningococcal isolates and possible implications for serogroup B vaccination. In: G. K. Schoolnik (Ed) The Pathogenic *Neisseria* (pp. 541–545). ASM, Washington, D.C.

Hawkes, R. E. Niday & J. Gordon (1982) A dot-immunobinding assay for monoclonal and other antibodies. Anal. Biochem. 119: 142–147

Mocca, L. F. & C. E. Frasch (1982) Sodium dodecyl sulfate-polyacrylamide gel typing system for characterization of *Neisseria meningitidis* isolates. J. Clin. Microbiol. 16: 240–244.

Peppler, M. S. & C. E. Frasch (1982) Proetection against group B *Neisseria meningitidis* disease: Effect of serogroup B polysaccharide and polymyxin B on immunogenicity of serotype protein preparations. Infect. Immun. 37: 264–270

Tsai, C-M, C. E. Frasch & L. F. Mocca (1981) Five structural classes of major outer membrane proteins in *Neisseria meningitidis*. J. Bacteriol. 146: 69–78

Wang, L Y. & C. E. Frasch (1984) Development of a *Neisseria meningitidis* group B serotype 2b protein vaccine and evaluation in a mouse model. Infect. Immun. 46: 408–414

Zollinger, W. D. & R. E. Mandrell (1983) Studies of the human antibody response to specific meningococcal outer membrane proteins of serotypes 2 and 15. Med. Tropicale 43: 143–147

Zollinger, W. D., R. E. Mandrell, J. M. Griffiss, P. Alteri & S. Berman (1979) Complex of meningococcal group B polysaccharide and type 2 outer membrane protein immunogenic in man. J. Clin. Invest. 63: 836–848

Zollinger, W. D., E. E. Moran, H. Connely, R. E. Mandrell & B. Brandt (1984) Monoclonal antibodies to serotype 2 and 15 outer membrane proteins of *Neisseria meningitidis* and their use in serotyping. Infect. Immun. 46: 260–266