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Monoclonal Antibodies to Serotype 2 and Serotype 15 Outer Membrane Proteins of *Neisseria meningitidis* and Their Use in Serotyping

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A series of murine monoclonal antibodies to serotype 2 and serotype 15 strains of *Neisseria meningitidis* were produced which were specific for outer membrane proteins of classes 1, 2, 3, and 5. A panel of eight monoclonal antibodies that exhibited a high degree of serotype specificity when reacted with prototype strains of known serotype were selected for study. Each of the corresponding epitopes was localized on a specific outer membrane protein by means of immunoprecipitation, electroblotting, or both. The serotype 2a-, 2b-, and 2c-specific antibodies bound to the class 2 protein, the serotype 15-specific antibody bound to the class 3 protein, two antibodies (3-1-P1.2 and 3-1-P1.16) bound to class 1 proteins, and two antibodies (1-1-P5.1 and 3-1-P5.2) bound to class 5 proteins. Six of these monoclonal antibodies were used in a spot-blot procedure to survey 122 case isolates (groups B, C, Y, and W135) and 363 carrier isolates (all serogroups) for the presence of the 2a, 2b, 2c, 15, P1.2, and P1.16 epitopes. A total of 66% of the case isolates and 30% of the carrier isolates reacted with one or more of the monoclonal antibodies. The use of monoclonal antibodies for serotyping of meningococci appears to be feasible and easy and appears to have significant advantages over the use of polyclonal typing sera.

Studies of meningococcal serotype antigens have been complicated by the presence of multiple surface antigens which show strain-to-strain variation. The lipopolysaccharide (LPS) (18, 22) and at least three major outer membrane proteins have been shown to exhibit antigenic variation (1, 9, 22, 27). Except for LPS typing, the antigens used for serotyping have been either the whole organism or vesicles of outer membrane referred to as outer membrane complex (OMC), outer membrane vesicles, or serotype antigen (6–8, 13, 26). In either case, multiple antigens with the potential for variability were present in these preparations. Since the polyclonal rabbit antisera used for serotyping were generally not monospecific nor characterized with respect to the particular outer membrane antigen(s) with which they reacted, it is not surprising that some confusion has arisen regarding the antigen(s) actually involved in serotyping reactions.

The major outer membrane proteins of the meningococcus have been classified on the basis of molecular weight, behavior on sodium dodecyl sulfate (SDS)-polyacrylamide gels, susceptibility to proteolytic enzymes, and peptide mapping into five different classes (24). The class 2 and class 3 proteins, however, appear to be functionally equivalent, and only one of the two is found on any given strain. Antigenic variability has been demonstrated for outer membrane proteins of classes 1, 2, 3, and 5 (1, 9, 22, 27), but only the class 2 (or 3) protein is usually referred to as the serotype protein. This is because the serotype 2 determinant, which was the first to be characterized, was shown by Frasch and Gotschlich to be located on the class 2 outer membrane protein (9), and this protein is more stable than the class 1 and class 5 proteins, which have been shown to exhibit considerable quantitative or qualitative (10, 20, 22) variation or both. Because of the inadequacies of conventional serotyping, however, the class 1 and class 5 proteins and also the LPS have been found to be involved in some of the protein serotype reactions (1, 21, 27). Although in some cases extensive absorptions have produced antisera that were in a practical sense monospecific (21), it is difficult to reproduce such antisera and to verify the resulting specificity.

The advent of monoclonal antibodies offers the opportunity to eliminate many of these problems and to greatly improve the accuracy and reproducibility of meningococcal serotyping. It also offers the opportunity to unify the nomenclature of serotyping and outer membrane antigens. Recently, a proposal has been made toward this end (C. E. Frasch, W. D. Zollinger, and J. T. Poolman, submitted for publication).

We report here the production of monoclonal antibodies to several outer membrane proteins of serotype 2 and serotype 15 strains. The particular outer membrane proteins to which these antibodies bind were determined, and a panel of these antibodies with defined specificities was used to survey both case strains and carrier strains isolated from U.S. Army personnel over the past 6 years for the presence of the corresponding epitopes.

MATERIALS AND METHODS

Bacterial strains. The prototype strains of *Neisseria meningitidis*, M986, B16B6, S3032, and H355, were obtained from Carl Frasch, Office of Biologics, U.S. Food and Drug Administration, Bethesda, Md.; strains 3006, 2996, 2369, and 3459 were obtained from Jan Poolman, University of Amsterdam, Amsterdam, The Netherlands; and strains 44/76, 111/79, 2729, 3042, 750225, and several additional strains from the Norwegian epidemic were obtained from L. Oddvar Froholm, National Institute of Public Health, Oslo, Norway. All other strains were from the culture collection of the Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, D.C. Cultures were stored in skim milk and were either lyophilized and sealed under vacuum or frozen at −70°C.

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Production of monoclonal antibodies. The methods used for production of monoclonal antibodies were described previously (28). The clone 1-1-P2a was obtained from a fusion of mouse spleen cells with the murine myeloma line P3-X63-AG8, and all others were obtained from fusions of mouse spleen cells with the nonproducer myeloma cell line X63-AG8.653 (16). Mice were immunized with two doses of live meningococci given both intravenously and intraperitoneally 1 to 4 months apart. The meningococcal strains used for vaccination included the following: 138I(C:2a:P1.2:P5.1:L3), 3006(B:2b:P1.2:P5.2:L2,3), 8214(Y:2c:P1.2:L?), H355(B:15:P1.15:P5.2:L3,8), and 44/76(B:15:P1.16:P5.2:L3,8). (In the preceding strain descriptions, the known antigenic determinants are given in parentheses in the following order: serogroup, protein serotype, class 1 protein determinants, class 5 protein determinants, and LPS type.) Clones were screened for the production of specific antibody with a solid-phase radioimmunoassay (25). After cloning twice in soft agar, the following clones were used for production of the monoclonal antibodies (ascites fluid) that were used in this study: 1-1-P2a, 3-1-P2b, 3-1-P1.2, 3-1-P1.16, 3-1-P5.2, 1-1-P5.1, 5-1-P2c, and 2-1-P15.

Immunoprecipitation. One-milligram samples of OMC were radioiodinated by the chloramine-T procedure (25) to a specific activity of ca. 200 cpm/ng of protein. Protein A Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) in 50-mg amounts was hydrated with 3 ml of solubilization buffer consisting of 0.05 M Tris-hydrochloride, 0.15 M NaCl, 0.01 M EDTA, and 1% Empigen BB (Albright and Wilson, Whitehaven, Cumbria, United Kingdom) (pH 7.8) and allowed to equilibrate for 1 h at room temperature. Unlabeled heterologous OMC containing 100 μg of protein was added to the Sepharose to cover nonspecific binding sites, and after 30 min of incubation, the Sepharose was removed by filtration through a glass fiber filter fitted in a 3-ml disposable syringe barrel. The protein A Sepharose was then washed four to five times with 3 ml of solubilization buffer.

The immunoglobulin fraction of mouse ascites fluid containing immunoglobulin G monoclonal antibody (2 to 10 mg/ml) was prepared by twice precipitating with ammonium sulfate at 40% of saturation. The final precipitate was dissolved in 0.05 M sodium phosphate buffer (pH 7.5). A portion of the antibody was diluted to 50 μg of protein per ml in solubilization buffer, and 1 ml was mixed with the washed protein A Sepharose. After mixing at room temperature for 30 min, the protein A Sepharose with bound monoclonal antibody was collected and washed five times with 3 ml of solubilization buffer as described above.

Samples of ^{125}I-labeled OMC (200 μl containing ca. 10^7 cpm) were diluted to a concentration of 1:10 in solubilization buffer. The mixture was sonicated for 1 min by holding the tube in a bath sonicator and was then placed in a boiling-water bath for 5 min. Any insoluble material was removed by centrifugation at 180,000 × *g* for 90 min at 22°C. The supernatant was collected and mixed with the monoclonal antibody-protein A Sepharose. After incubation for 1 h at room temperature, the protein A Sepharose was collected, washed with solubilization buffer as described above, and mixed with ca. 300 μl of 2× SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer (4% SDS, 20% glycerol, 0.01% bromphenol blue, 0.12 M Tris-hydrochloride [pH 6.8]). This mixture was heated for 5 min in a boiling-water bath, and the Sepharose was removed by centrifugation. The supernatant which contained 500 to 2,000 cpm/μl was analyzed by SDS-PAGE.

Electrophoresis and electroblotting. SDS-PAGE was performed by the method of Laemmli (17), sometimes with the addition of urea (2 to 4 M final concentration) to the stacking and resolving gel buffers.

Electroblotting was done after the Western blot procedure of Burnette (3), except the blocking buffer contained 2% casein rather than 5% bovine serum albumin.

Autoradiography of nitrocellulose papers or dried gel slabs was done with Kodak X-Omatic cassettes with regular intensifying screens and Kodak X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.). Films were exposed at −70°C.

Specific bands to which antibody bound were identified on the autoradiogram by reference to controls, which consisted of ^{125}I-labeled OMC of the same or a related strain. Controls were reacted with monoclonal antibody and [^{125}I]protein A along with the unlabeled samples. Since the amount of protein applied to the control lanes was very low compared with that in the sample lanes, there was negligible binding of monoclonal antibody and hence labeled protein A to the controls.

Serotyping. With the exception of a few strains which were typed by staphylococcal A coagglutination (12), serotyping was done by a spot-blot method with nitrocellulose paper (4, 14). In the spot-blot procedure, cells from an overnight culture on nutrient agar were used to prepare a heavy saline suspension, and a few microliters of the suspension were applied in a small spot on each of several nitrocellulose papers (one for each monoclonal antibody to be used). A flexible microtiter plate with wells removed was used as a template. The sheets were allowed to dry and further processed as described above for electroblotting. Ascites fluid diluted to a concentration of 1:500 to 1:10,000 in blocking buffer (0.01 M Tris-hydrochloride, 0.9% NaCl, 0.02% sodium azide, 2% casein [pH 7.4]) was used as the source of monoclonal antibody. The dilution used was the highest dilution that gave maximum spot intensity. Depending on the isotype of the monoclonal antibody, either [^{125}I]protein A or ^{125}I-labeled rabbit anti-mouse immunoglobulin was used to detect the amount of monoclonal antibody bound. Autoradiography was done as described above.

RESULTS

Specificity of monoclonal antibodies. Monoclonal antibodies were initially selected based on their binding in a solid-phase radioimmunoassay to OMC from a panel of three to six strains of known serotype. The specificity of the antibodies was further defined by reacting them with a larger panel of strains by the spot-blot serotyping procedure (Table 1). The specificity of the monoclonal antibodies fell into three groups. The first group exhibited clear serotype specificity as judged by the pattern of reactions with strains of known serotype. This group included those antibodies with specificities 2a, 2b, 2c, and 15. A second group appeared to have broader specificities which appeared to correlate with certain serotypes. This group included the antibodies with P1.2 and P1.16 specificities. The antibody with P1.2 specificity reacted with most strains of serotypes 2a, 2b, and 2c and also with strain 126E, which is the prototype strain for serotype P3 (26) and factor III described by Gold and Wyle (13). The antibody with P1.16 specificity reacted with serotype 16 and most serotype 15 strains of Norwegian origin. A third group (P5.1 and P5.2) exhibited specificities that did not appear to correlate with serotype.

These results demonstrated the feasibility of producing

TABLE 1. Specificity of monoclonal antibodies to meningococcal outer membrane proteins

Serotype test strains		Monoclonal antibody specificity							
Type	Strain	2a	2b	2c	P1.2	15	P1.16	P5.1	P5.2
2a	138I	+	−	−	+	−	−	+	−
	M986	+	−	−	+	−	−	−	−
	B16B6	+	−	−	+	−	−	−	−
	99M	+	−	−	+	−	−	+	−
2b	3006	−	+	−	+	−	−	−	+
	2996	−	+	−	+	−	−	−	−
	8047	−	+	−	+	−	−	−	−
2c	2369	−	−	+	+	−	−	−	−
	3459	−	−	+	+	−	−	−	−
15	44/76	−	−	−	−	+	+	−	+
	P355	−	−	−	−	+	−	−	+
16	2729	−	−	−	−	−	+	−	−
	3042	−	−	−	−	−	+	−	−
	750255	−	−	−	−	−	+	−	+

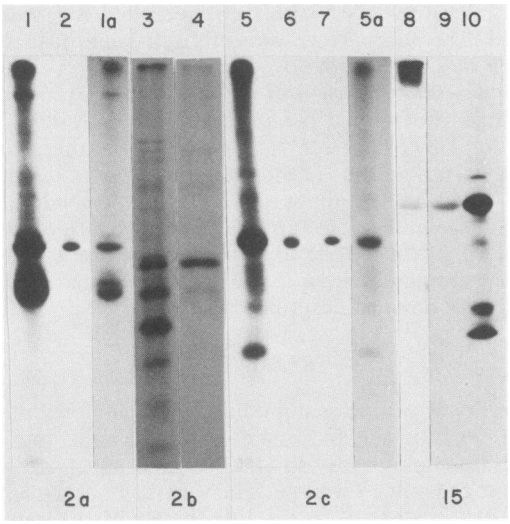

FIG. 1. Reaction of monoclonal antibodies with class 2 and 3 meningococcal outer membrane proteins. Lanes 1 to 7 are from autoradiograms of dried gel slabs after SDS-PAGE of ^{125}I-labeled outer membrane protein precipitated with monoclonal antibody or of ^{125}I-labeled OMC controls. Lanes 8 to 10 are an autoradiogram of a Western blot sequentially reacted with the monoclonal antibody 2-1-P15 and [^{125}I]protein A. Lanes 1 and 1a, ^{125}I-labeled 99M(B:2a:P1.2:P5.1:L3) OMC after 4 and 1 h of exposure, respectively; lane 2, 99M protein precipitated with antibody 1-1-P2a; lane 3, ^{125}I-labeled 8047(B:2b:P1.2:L3,4,6) OMC; lane 4, ^{125}I-labeled 8047 protein precipitated with the monoclonal antibody 3-1-P2b; lanes 5 and 5a, ^{125}I-labeled 8214(Y:2c:P1.2:L?) OMC after 4 and 1 h of exposure, respectively; lane 6, ^{125}I-labeled 8214 protein precipitated with the monoclonal antibody 5-1-P2c; lane 7, ^{125}I-labeled 8214 protein precipitated with the monoclonal antibody 3-1-P1.2; lane 8, H355(B:15:P1.15:P5.2:L3,8) OMC solubilized with 2% SDS at room temperature; lane 9, H355 OMC solubilized with 2% SDS at 100°C for 10 min; and lane 10, ^{125}I-labeled H355 OMC.

monoclonal antibodies with clear serotype specificity which could be used as serotyping reagents and in studies of the serotype proteins.

Localization of the serotype-specific epitopes on the outer membrane proteins. Both immunoprecipitation and electroblotting techniques were used to determine the specific outer membrane protein to which each of the monoclonal antibodies bound. It was generally found that monoclonal antibodies directed toward epitopes on the class 1 and class 5 proteins were able to bind to those proteins after SDS-PAGE and electroblotting, whereas antibodies directed towards the class 2 or 3 protein could no longer bind after SDS-PAGE. It was therefore necessary to use immunoprecipitation methods to demonstrate binding to class 2 and class 3 proteins. The class 3 serotype 15 protein was an exception in that some monoclonal antibody still bound after denaturation by SDS.

The monoclonal antibodies with specificities 2a, 2b, 2c, and 15 all bound to the class 2 or class 3 principal outer membrane protein (Fig. 1) and were therefore considered to have true serotype specificity. Serotype 2a-specific monoclonal antibodies from five different clones were tested for the capacity to bind to the serotype protein after SDS-PAGE and electroblotting. All were able to bind to the protein in the aggregated form (sample dissolved in 2% SDS at room temperature), but none were able to bind to the monomeric form (sample boiled 10 min in 2% SDS). Immunoprecipitation with the 2a, 2b, and 2c antibodies resulted in a substantial purification of the respective class 2 protein, but the immunoprecipitates were not completely free of contamination by other closely associated outer membrane proteins, such as the class 1 and class 4 proteins (Fig. 1, lane 4). The class 1 and class 2 proteins of serotype 2c strains were not well resolved by SDS-PAGE in the Laemmli gel system with or without urea, and consequently, only a very slight difference was seen in the position of the labeled proteins that were precipitated with the 2c- and P1.2-specific monoclonal antibodies (Fig. 1, lanes 6 and 7). The relatively weak binding of the serotype 15 monoclonal antibody to the class 3 serotype protein after SDS-PAGE and electroblotting is shown in lanes 8 and 9. Much stronger binding of the antibody to the aggregated form of the protein in the unboiled sample is evident in lane 8.

Binding of the monoclonal antibodies with P1.2 and P1.16 specificities to OMC after SDS-PAGE and electroblotting is shown in Fig. 2. In addition to binding to the class 1 protein, a variable amount of binding to a second lower-molecular-weight band was sometimes observed (lanes 1, 2, 3, 6, and 9). In the case of strain 3459 (lane 2), the second band had a molecular weight of ca. 40,000 as compared with ca. 30,000 for lanes 1, 3, 6, and 9. The binding to the higher-molecular-weight band was consistent, whereas the presence of the lower-molecular-weight band was variable and dependent on how the sample was handled before electrophoresis. The possibility that two different monoclonal antibodies were present was ruled out by repeating the experiment after recloning the hybridomas. Anti-P1.2 antibody bound to the class 1 protein of prototype 2a, 2b, and 2c strains as well as to the serotype P3 prototype strain, 126E. Small differences in molecular weight of the class 1 protein in these strains did not appear to affect the antigenic specificity of this epitope. The P1.16-specific monoclonal antibody bound to the class 1 protein of several type 15 test strains from Norway but not to the prototype strain H355, which has a different class 1 protein (P1.15) than 44/76. It also bound to the class 1 protein of the serotype 12 strain, S-3032.

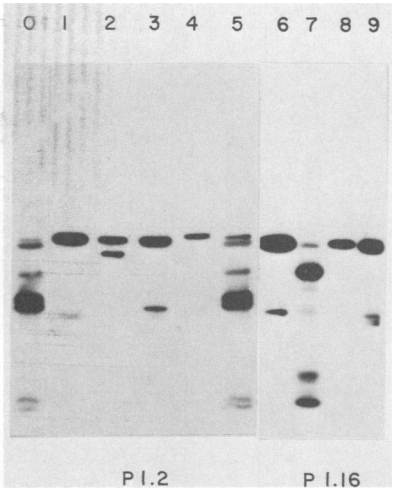

FIG. 2. Western blots showing reaction of class 1 meningococcal outer membrane proteins with two different monoclonal antibodies. Lanes 1 to 5 were reacted with antibody 3-1-P1.2, and lanes 6 to 9 were reacted with antibody 3-1-P1.16. ^{125}I-labeled protein A was used to detect the bound antibody. Lane 0, ^{125}I-labeled 3006(B:2b:P1.2:P5.2:L2,3) OMC; lane 1, 3006 OMC; lane 2, 3459(NG:2c:P1.2:L?) OMC; lane 3, 126E(C:3:P1.2:L1,8) OMC; lane 4, 138I(C:2a:P1.2:P5.1:L3) OMC; lane 5, ^{125}I-labeled 3006 OMC; lane 6, 44/76(B:15:P1.16:P5.2:L3,8) OMC; lane 7, ^{125}I-labeled H355(B:15:P1.15:P5.2:L3,8) OMC; lane 8, 111/79(B:15:P1.16:L?) OMC; and lane 9, S3032(B:12:P1.16:L3) OMC.

The monoclonal antibodies with specificity for the P5.1 and P5.2 epitopes bound to the heat-modifiable class 5 proteins (Fig. 3). Binding to both forms of the proteins was evident but was stronger to the heat-modified form.

Serotyping with monoclonal antibodies. A total of 125 case strains and 363 carrier strains isolated from U.S. Army personnel between 1978 and 1983 were tested for their capacity to react with monoclonal antibodies specific for the determinants 2a, 2b, P1.2, 15, and P1.16. The case strains were also tested for the presence of the 2c determinant.

Among the case isolates surveyed, all but a few of those that were positive were serotype 2 related (Table 2). Overall, 62% of the case isolates of groups B, C, Y, and W135 were positive for one of the six determinants. Many of the strains with the 2a, 2b, or 2c determinant on the class 2 (serotype) protein also had the P1.2 determinant on the class 1 protein. The 2b determinant occurred mostly among the group B strains, whereas the 2a and 2c determinants occurred mostly among the group Y and W135 strains. The small sample of group C strains were a mixture of serotypes 2a, 2b, and 2c. Over 80% of the group Y case strains were serotype 2 related. Most of these were serotype 2a, but eight were negative for 2a, 2b, and 2c and positive for P1.2.

A relatively high percentage (65%) of group B case strains isolated from active-duty military personnel (mostly recruits) were positive for one of the six determinants. Among these group B strains were a relatively large number of serotype 2b strains. Contributing to this total were 12 strains isolated at Ft. Benning, Ga., between January 1982 and September 1983. These 12 strains were all positive for both the 2b and P1.2 determinants. This small group of strains, which appeared homogeneous with respect to the class 1 and class 2 proteins, were also tested for the presence of epitopes P5.1 and P5.2. Only 1 of 12 strains was positive for the P5.1 epitope, and 5 of 12 were positive for the P5.2 epitope.

The proportion of carrier strains that were positive for one of the five epitopes included in the survey (31%) was considerably lower than for the case strains, especially for groups B and C (Table 3). Serotype 15-related strains were more common than serotype 2-related strains. In fact, only ca. 3% of the strains had the serotype 2a or 2b determinants, and ca. 9% had the P1.2 determinant.

The two type-15 related epitopes, 15 and P1.16, were seldom found on the same strain. The occurrence of these five epitopes was as high among the rough, nongroupable, multiagglutinable strains as among the groupable strains.

DISCUSSION

Hybridoma monoclonal antibodies appear to have excellent possibilities for use in meningococcal serotyping, serogrouping (28), and other studies of cell surface antigens. Some initial concerns that monoclonal antibodies would be too specific appear to be unfounded. The monoclonal antibodies used in this study were obtained from fusions involving spleen cells from mice which were immunized with viable meningococci. These antibodies, which bound to the class 2 or class 3 protein, were found to have specificities which closely paralleled the dominant specificity of the polyvalent typing sera used to define the presently recognized serotypes, 2a, 2b, 2c, and 15. It is likely that if the animals were immunized with purified or partially purified outer membrane proteins, many of the resultant antibodies to the serotype proteins would show some degree of cross-reaction due to exposure of conserved regions of the polypeptide chains not normally exposed on the intact organism.

The localization of the serotype 2a, 2b, 2c, and 15 epitopes on the class 2 or class 3 outer membrane protein is consistent with the findings of Frasch and Gotschlich (9) and of Poolman et al. (21) and with the designation of that protein

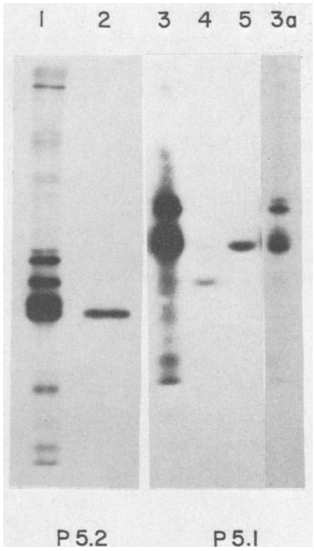

FIG. 3. Reaction of class 5 meningococcal outer membrane proteins with the monoclonal antibodies 3-1-P5.2 and 1-1-P5.1. Lane 1, ^{125}I-labeled 3006(B:2b:P1.2:P5.2:L2,3) OMC; lane 2, ^{125}I-labeled 3006 protein precipitated with the monoclonal antibody 3-1-P5.2; lanes 3 and 3a, Western blot of ^{125}I-labeled 99M(B:2a:P1.2:P5.1:L3) OMC after 4 and 1 h of exposure, respectively; lane 4, Western blot of 99M OMC solubilized with 2% SDS at room temperature; and lane 5, 99M OMC solubilized with 2% SDS for 10 min at 100°C. The nitrocellulose paper (lanes 3 to 5) was reacted with the monoclonal antibody 1-1-P5.1.

TABLE 2. Prevalence of serotype 2 and serotype 15 related epitopes on case isolates of meningococci from U.S. military personnel, 1978–1983

Serogroup	Isolate source	No. of strains tested	No. of strains reacting with monoclonal antibody or antibodies of indicated specificity											
			2a	2b	15	P1.2	P1.16	2a + P1.2	2b + P1.2	2c + P1.2	15 + P1.16	2a + P1.16	NR[a]	% Positiv
B	Active duty	26		1					15		1		9	65
	Dependents	29		4	1	1	1		10				12	59
C	Active duty	3						1	1				1	67
	Dependents	6						1	2	1			2	67
Y	Active duty	29				8		14	1	3		1	2	93
	Dependents	6						4					2	67
W135	Active duty	18	4			1		2					11	39
	Dependents	5				2	1						2	60
Total		122	4	5	1	12	2	22	29	4	1	1	41	66

[a] NR, No reaction with any of the monoclonal antibodies.

as the serotype protein. There remains some ambiguity in the case of the 2c determinant, since the class 1 and class 2 proteins were not well resolved by SDS-PAGE. We concluded that the 2c and P1.2 epitopes were on different proteins, based on a slight difference in electrophoretic mobility of the two immunoprecipitates and by analogy with the 2a and 2b strains. The monoclonal antibody specific for the P1.2 epitope bound to the class 1 protein of many, but not all, 2a, 2b, and 2c strains. This shared epitope helps to explain the cross-reactions observed between these strains (21). This antibody also bound to the class 1 protein of the group C strain 126E, which is the prototype strain for the P3 specificity previously defined in the solid-phase radioimmunoassay inhibition serotyping system (26). It is probable that most of the strains that were serotyped as P3 in that study were positive as a result of the presence of the P1.2 epitope. The anti-P1.16 antibody bound to the class 1 protein of the serotype 12 prototype strain, S-3032, as well as the serotype 15 strain, 44/76. This is consistent with the report by Holten (15) of cross-reactivity between these two strains.

The task of determining the particular outer membrane protein to which a monoclonal antibody binds is complicated by the fact that some of the proteins, especially the class 2 and 3 proteins, lose antigenic activity when converted to the monomeric state by boiling in SDS before SDS-PAGE and electroblotting. Proteins that behave in this manner also present difficulties in the immunoprecipitation method, because conditions which completely dissociate the proteins also tend to denature them. The observation that renaturation of the class 2 protein with the dipolar ionic detergent Empigen BB is sometimes possible (19) may be helpful in resolving this dilemma. This observation enabled us to confirm by electroblotting the results obtained by immunoprecipitation in the present study (19).

During the preparation of samples for electrophoresis and electroblotting, we observed what appeared to be a partial breakdown of several of the class 1 proteins to antigenically active, smaller peptides (Fig. 2). This phenomenon was not studied in detail, but one might speculate that it resulted from enzymatic cleavage of this protein by an endogenous protease.

The use of monoclonal antibodies for serotype analysis permits one to study the antigenic variation that occurs in each class of outer membrane proteins separately. In the case of group B strains, this type of information is important in formulating a vaccine based on the outer membrane proteins and in assessing the epidemiological significance of each protein. The number of antigenically different proteins within each class of outer membrane protein is unknown, but a comparison of the degree of variability of each would be useful.

Once the monoclonal antibodies are produced and charac-

TABLE 3. Prevalence of serotype 2 and serotype 15 related epitopes on carrier strains of meningococci isolated at Ft. Dix, N.J., 1978–1982

Serogroup	No. of strains	No. of strains reacting with monoclonal antibody or antibodies of indicated specificity									
		2a	15	P1.2	P1.16	15 + P1.16	2a + P1.2	2b + P1.2	15 + P1.2	NR[a]	% Positive
B	99[b]	4		9	8			2		76	23
B	32[c]			1	5	1				25	22
C	13[c]							1		12	8
29E	15[c]			2	3					10	33
W135	22[c]			3	9					10	55
X	43[c]	1	5		4					33	23
Y	6[c]			2			3			1	83
Z	1[c]									1	0
R, NG, MA[a]	132[c]		25	4	9	5	1		3	85	36
Total	363	5	30	21	38	6	4	3	3	253	30

[a] Abbreviations: NR, no reaction with any of the monoclonal antibodies; R, rough (agglutinated in saline); NG, no agglutination in saline or any of the grouping sera; MA, no agglutination in saline but agglutination in more than one of the grouping sera.

[b] Strains isolated 1978–1981.

[c] Strains isolated 1982.

terized, serotyping may be performed by any of a variety of methods. We compared the staphylococcal A coagglutination method and the spot-blot method and found that the spot-blot method gave the most reliable results. This test is very similar to the filter radioimmunoassay described by De Marie et al. (4), except the organisms are not grown on the paper.

Although the epidemic of group B meningococcal disease that occurred in U.S. military recruits during the early 1960s was predominantly due to serotype 2a strains, no 2a strains were present among the case isolates from U.S. Army personnel during the 1978–1983 period. There were only four strains with the 2a epitope found among 131 group B carrier strains isolated during the same period. Serotype 2b strains, on the other hand, accounted for over half of the case strains isolated during the 1978–1982 period. A high prevalence of serotype 2b strains among group B cases in recent years has also been reported in Canada (1), The Netherlands (22), and South Africa (W. D. Zollinger, R. E. Mandrell, H. Connolly, and E. E. Moran. International Conference on Pathogenic *Neisseria*, Montreal, Canada, abstr. no. 61, 1982). This is in contrast to the high prevalence of serotype 15 strains in Norway (2, 12, 15) and the heterogeneity of serotypes among group B strains from Spain and several other countries (23). Although the predominant group B serotype causing systemic disease in the U.S. Army has changed from 2a to 2b, the presence of the P1.2 epitope on the class 1 protein has remained constant. The 2a epitope has not altogether disappeared, however, since ca. 88% of the group Y case isolates carried this epitope.

The most frequently occurring epitopes on the carrier strains were P1.16 and 15, but they were seldom found together on the same strain. By comparison, most of the recent Norwegian type 15 case strains carried both the 15 and the P1.16 determinants (2, 12). The relatively frequent occurrence of epitopes 15 and P1.16 on carrier strains suggest that neither of these epitopes by itself is a virulence marker. In the case of the Norwegian strains, however, their occurrence together may be a virulence marker. A relatively high percentage of group Y and W135 carrier strains reacted with at least one of the monoclonal antibodies. Three of six group Y strains were serotype 2a, but only the class 1 epitopes P1.2 and P1.16 were identified on the group W135 strains.

Based on the serotyping of group B case strains isolated from U.S. Army personnel over the past 5 years, a vaccine based on the major outer membrane proteins (5, 11, 29, 30) and intended for use in this population should probably be prepared from a strain having the 2b and P1.2 determinants. Such a vaccine, if fully effective, might have provided protection against 60 to 70% of the group B disease that occurred during that time. This is probably higher than would be expected for a strictly endemic situation, since a small outbreak of group B serotype 2b disease occurred at Ft. Benning, Ga., during 1982 and 1983.

LITERATURE CITED

1. **Ashton, F. E., J. A. Ryan, C. Jones, B. R. Brodeur, and B. B. Diena.** 1983. Serotypes of *Neisseria meningitidis* associated with an increased incidence of meningitis cases in the Hamilton area, Ontario, during 1978 and 1979. Can. J. Microbiol. **29**:129–136.
2. **Bovre, K., L. O. Froholm, P. Gaustad, E. Holten, and E. A. Hoiby.** 1983. Some agent characteristics and their coexistence related to occurrence and severity of systemic meningococcal disease in Norway, Winter 1981–1982. NIPH Ann. **6**:75–84.
3. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
4. **De Marie, S., J. H. J. Hoeijmakers, J. T. Poolman, and H. C. Zanen.** 1981. Epidemiology of *Neisseria meningitidis*: serogrouping and serotyping; a method for large-scale serotyping. Antonie van Leeuwenhoek J. Microbiol. **47**:470–472.
5. **Frasch, C. E.** 1977. Role of protein serotype antigens in protection against disease due to *Neisseria meningitidis*. J. Infect. Dis. **136**(Suppl.):84–90.
6. **Frasch, C. E.** 1979. Noncapsular surface antigens of *Neisseria meningitidis*, p. 304–337. *In* L. Weinstein and B. N. Fields (ed.), Seminars in infectious disease, vol. 2. Stratton Intercontinental Medical Book Corp., New York.
7. **Frasch, C. E., and S. S. Chapman.** 1972. Classification of *Neisseria meningitidis* group B into distinct serotypes. I. Serological typing by a microbactericidal method. Infect. Immun. **5**:98–102.
8. **Frasch, C. E., and S. S. Chapman.** 1972. Classification of *Neisseria meningitidis* group B into distinct serotypes. II. Extraction of type-specific antigens for serotyping by precipitin techniques. Infect. Immun. **6**:127–133.
9. **Frasch, C. E., and E. C. Gotschlich.** 1974. An outer membrane protein of *Neisseria meningitidis* group B responsible for serotype specificity. J. Exp. Med. **140**:87–104.
10. **Frasch, C. E., and L. F. Mocca.** 1978. Heat-modifiable outer membrane proteins of *Neisseria meningitidis* and their organization within the membrane. J. Bacteriol. **136**:1127–1134.
11. **Frasch, C. E., M. S. Peppler, T. R. Cate, and J. M. Zahradnik.** 1981. Immunogenicity and clinical evaluation of group B *Neisseria meningitidis* outer membrane protein vaccine, p. 263–267. *In* J. B. Robbins, J. C. Hill, and J. C. Sadoff (ed.), Seminars in infectious disease, vol. 4. Bacterial vaccines. Thieme-Stratton Inc., New York.
12. **Froholm, L. O., K. Bovre, E. Holten, and W. D. Zollinger.** 1983. Serotyping of meningococci by coagglutination with monoclonal antibodies. NIPH Ann. **6**:125–131.
13. **Gold, R., and F. A. Wyle.** 1970. New classification of *Neisseria meningitidis* by means of bactericidal reactions. Infect. Immun. **1**:479–484.
14. **Hawkes, K. R., E. Niday, and J. Gordon.** 1982. A dot-immunobinding assay for monoclonal and other antibodies. Anal. Biochem. **119**:142–147.
15. **Holten, E.** 1979. Serotypes of *Neisseria meningitidis* isolated from patients in Norway during the first six months of 1978. J. Clin. Microbiol. **9**:186–188.
16. **Kearney, J. F., A. Radbruch, B. Liesegang, and K. Rajewsky.** 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. **123**:1548–1550.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Mandrell, R. E., and W. D. Zollinger.** 1977. Lipopolysaccharide serotyping of *Neisseria meningitidis* by hemagglutination inhibition. Infect. Immun. **16**:471–475.
19. **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 protein. J. Immunol. Methods **67**:1–11.
20. **Poolman, J. T., S. De Marie, and H. C. Zanen.** 1980. Variability of low-molecular-weight, heat-modifiable outer membrane proteins of *Neisseria meningitidis*. Infect. Immun. **30**:642–648.
21. **Poolman, J. T., C. T. P. Hopman, and H. C. Zanen.** 1980. Immunochemical characterization of *Neisseria meningitidis* serotype antigens by immunodiffusion, and SDS-polyacrylamide gel electrophoresis immunoperoxidase techniques and the distribution of serotypes among cases and carriers. J. Gen. Microbiol. **116**:465–473.
22. **Poolman, J. T., C. T. P. Hopman, and H. C. Zanen.** 1982. Problems in the definition of meningococcal serotypes. FEMS

Lett. **13**:339–348.
23. **Saez-Nieto, J. A., B. B. Barreno, C. L. Galindez, and J. Casal.** 1981. Meningitis meningococica en Espana (1978–1980). II. Serotipos y patrones electroforeticos en gel de poliacrilamida. Rev. Sanid. Hig. Publica **55**:1295–1308.
24. **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.
25. **Zollinger, W. D., J. M. Dalrymple, and M. Artenstein.** 1976. Analysis of parameters affecting the solid phase radioimmunoassay quantitation of antibody to meningococcal antigens. J. Immunol. **117**:1788–1798.
26. **Zollinger, W. D., and R. E. Mandrell.** 1977. Outer-membrane protein and lipopolysaccharide serotyping of *Neisseria meningitidis* by inhibition of a solid-phase radioimmunoassay. Infect. Immun. **18**:424–433.
27. **Zollinger, W. D., and R. E. Mandrell.** 1980. Type-specific antigens of group A *Neisseria meningitidis*: lipopolysaccharide and heat-modifiable outer membrane proteins. Infect. Immun. **28**:451–458.
28. **Zollinger, W. D., and R. E. Mandrell.** 1983. Importance of complement source in bactericidal activity of human antibody and murine monoclonal antibody to meningococcal group B polysaccharide. Infect. Immun. **40**:257–264.
29. **Zollinger, W. D., R. E. Mandrell, and J. M. Griffiss.** 1982. Enhancement of immunologic activity by noncovalent complexing of meningococcal group B polysaccharide and outer membrane proteins, p. 254–262. *In* J. B. Robbins, J. C. Hill, and J. V. Sadoff (ed.), Seminars in infectious disease, vol. 4. Bacterial vaccines. Thieme-Stratton Inc., New York.
30. **Zollinger, W. D., R. E. Mandrell, J. M. Griffiss, P. Altieri, and S. Berman.** 1979. Complex of meningococcal group B polysaccharide and outer membrane protein immunogenic in man. J. Clin. Invest. **63**:836–848.