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Development and Characterization of an Anti-Idiotype Antibody to the Capsular Polysaccharide of *Neisseria meningitidis* Serogroup C

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A monoclonal anti-idiotypic antibody (Ab2) whose antibody combining site contained a surrogate image of the meningococcal group C capsular polysaccharide was developed. To accomplish this, a monoclonal antibody against the group C capsular polysaccharide was developed by the fusion of splenocytes from mice immunized with *Neisseria meningitidis* group C strain MP13 with Sp2/0-Ag14 plasmacytoma cells. Monoclonal antibody 1E4, an immunoglobulin M isotype, demonstrated binding to the serogroup C polysaccharide in enzyme-linked immunosorbent assay (ELISA). Monoclonal antibody 1E4 reacted with 30 of 30 group C strains and 1 of 36 group B strains in immunodot assay, slide agglutination, inhibition ELISA, and bactericidal assay. This monoclonal antibody was selected as idiotype (Ab1) for the development of hybridomas producing an anti-idiotype antibody. One of the hybridomas developed, designated 6F9, was capable of over 70% inhibition of 1E4 in binding in the meningococcal C polysaccharide-specific ELISA. Studies with convalescent human serum demonstrated 100% inhibition of a serogroup C-specific ELISA with 200 μg of 6F9 per ml and 50% inhibition of this ELISA was achieved with 50 μg of 6F9 per ml. Monoclonal anti-anti-idiotype antibodies (Ab3) with specificities similar to Ab1, 1E4 were generated from BALB/c mice immunized with the Ab2 (6F9). Immunization of rabbits with 6F9 resulted in an immunoglobulin G response which was significantly greater than that of control to a titer of 1:160. These studies indicate that monoclonal 6F9 contained a surrogate image on the combining antibody site which mimicked meningococcal C polysaccharide. This surrogate image is capable of evoking antibodies to the meningococcal C polysaccharide in syngenic and xenogenic species.

Meningococcal disease is a major cause of morbidity and mortality among children and adults throughout the world (6, 37). A yearly incidence of 2,000 to 3,000 cases is reported in the United States, with a peak attack rate among infants at 4 to 6 months of age (6, 37). Although nine distinct serogroups of meningococci are identified, groups B and C account for approximately 70 to 80% of disease under endemic conditions. Despite the dramatic impact of antibiotics on the case fatality rate, the attack rates for infection have changed little, and the disease still has a significant case fatality rate ranging from 7 to 15% in industrialized countries (1). The emergence of sulfonamide-resistant strains of meningococci and miniepidemics in military recruits led to the development of meningococcal polysaccharide vaccines (18). The vaccines are highly immunogenic and effective in adults (4, 20), but the seroresponse in infants is minimal and of short duration (15, 31, 34). The lack of immune response in children under 2 years old is not limited to the meningococcal polysaccharide vaccine. Similar poor antibody responses to immunizations with other polysaccharide vaccines such as *Haemophilus influenzae* type b and some capsular serotype polysaccharides of *Streptococcus pneumoniae* have been observed (11, 38). The immune response to polysaccharide antigens has several characteristics which distinguish it from the antibody response to protein antigens. These characteristics include thymus independence (35), failure to stimulate a memory response and to undergo affinity maturation (i.e., lack of booster response) (24), and late development in ontogeny (15, 24). Efforts to overcome the neonatal unresponsiveness to polysaccharide antigens have resulted in vaccines in which the polysaccharide capsular antigens are changed into functional T-cell-dependent antigens by coupling the polysaccharides to an immunogenic thymus-dependent carrier (7, 9, 41). An alternative strategy for the conversion of the thymus-independent polysaccharide into a thymus-dependent immunogen is the development of the surrogate image of the polysaccharide in the combining site of an anti-idiotype antibody (28). This protein image of a polysaccharide antigen can be easily manipulated and coupled to potent immunogenic carriers to become T-cell-dependent antigens which can receive full T-cell help.

In this report, we describe the production of a monoclonal antibody (Ab1) which recognized the capsular polysaccharide of *Neisseria meningitidis* group C and the development of a monoclonal anti-idiotypic antibody (Ab2) which mimicked the capsular polysaccharide of *N. meningitidis* group C. Ab2 was capable of evoking antibodies (Ab3) in rabbits and mice which were specific for meningococcal C polysaccharide and, in the presence of complement, were bactericidal.

MATERIALS AND METHODS

Animals. BALB/c mice were obtained from West Seneca Laboratories (West Seneca, N.Y.). Rabbits were obtained from Beckens Animal Farms (Sanborn, N.Y.).

Bacterial strains. Eighty strains of *N. meningitidis* were obtained from our own collection. These included 30 group C strains, 34 group B strains, 6 group A strains, 6 group Y strains, 2 group W135 strains, and 2 group X strains. Strains were grown on supplemented GC agar (Difco Laboratories, Detroit, Mich.) in 5% CO_2 at 37°C. To confirm the serogroup of each strain, all organisms were typed with typing sera from the Centers for Disease Control prior to use in the experiments. Isolates were stored in Mueller-Hinton broth

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(Difco) with 10% glycerol at −70°C. *N. meningitidis* serogroup C strain MP13 was used as immunogen for the development of Ab1. This strain produces a capsular C polysaccharide which lacks *O*-acetyl groups (2).

Antisera. Rabbit anti-meningococcal C polysaccharide typing antisera and rabbit anti-meningococcal A polysaccharide typing antisera were obtained from the Centers for Disease Control. Human serum containing antibodies to meningococcal C polysaccharide was obtained from a patient convalescing from group C meningococcal infection. Monoclonal antibody 3E4 (immunoglobulin G2b [IgG2b] isotype) which was used as a control in enzyme-linked immunosorbent assay (ELISA) inhibition and fluorescence studies is an anti-idiotypic antibody which contains a region on its combining site which mimics the group A meningococcal capsular polysaccharide (A. Campagnari, M. Wirth, M. A. J. Westerink, G. Muller, and M. Apicella, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, E-58, p. 112).

Preparation of group C meningococcal polysaccharide. The capsular polysaccharide from *N. meningitidis* serogroup C was prepared from strains MP13 and MP1 by the technique described by Gotschlich (19). Vaccine-quality group A and group C meningococcal capsular polysaccharides were gifts from Merck Sharp & Dohme Research Laboratories (Rahway, N.J.).

Development of monoclonal antibodies. Monoclonal antibodies against group C capsular polysaccharide (the idiotype or Ab1) were prepared by immunizing BALB/c mice intraperitoneally with 0.3 cm^3 of heat-killed (10 min at 100°C) whole organism suspension of 10^8 organisms per ml in phosphate-buffered saline (PBS) of strain MP13 on days 0 and 28. On day 32, the spleens were removed, and the splenocytes were fused to NS1 (variant of IgG1 BALB/c plasmacytoma P3xAg8) plasmacytoma cells. The fusion procedure and media used were as described by Kennett (27). Supernatants from the initial plating in 96-well plates were screened for production of antibody by immunodot assay by using purified capsular polysaccharide and whole cell preparation of MP13 as antigens on nitrocellulose. Cells from wells strongly positive for capsular polysaccharide were cloned by limiting dilution. Large quantities of antibody were produced by intraperitoneal injection of hybridoma cells into pristane-treated BALB/c mice (14). After an incubation period of 3 weeks, the ascitic fluid containing high concentrations of antibodies was harvested. The spleens of these ascitic mice were removed and used for the production of monoclonal anti-idiotype antibodies (Ab2). The splenocytes were fused with SP 2/0 Ag14 plasmacytoma cells according to standard procedure (27). Supernatants from the 96-well plates were screened initially for production of anti-idiotype antibody by immunodot assay using the Ab1 monoclonal antibody as antigen. The positive hybridomas were cloned by limiting dilution and studied as described in the serological methods. The monoclonal antibodies were isotyped by using a mouse monoclonal isotyping kit (Hyclone Laboratories, Logan, Utah). Large quantities of Ab1 free of other large contaminating proteins were obtained by growing the hybridoma cells in dialysis membranes (43). The antibodies were purified by P300 molecular-sieve chromatography (Bio-Rad Laboratories, Richmond, Calif.).

Monoclonal anti anti-idiotype antibodies (Ab3) were prepared by immunizing pristane-treated BALB/c mice intraperitoneally with 10^5 hybridoma cells secreting anti-idiotypic antibody Ab2. On day 21, the spleens were removed and splenocytes were fused with SP 2/0 Ag14 plasmacytoma cells by standard procedure (27). Supernatants from the 96-well plates were screened for production of anticapsular antibody (Ab3) by immunodot assay using purified capsular polysaccharide as antigen. The positive hybridomas were cloned by limiting dilution. Large quantitites of antibody were produced by intraperitoneal injection of hybridoma cells into pristane-treated mice (14).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by the modified method of Laemmli (30). Gels were either silver stained or stained with Coomassie blue (48). Western blot (immunoblot) was performed by the method of Towbin et al. (10, 47).

Immunodot assay. Immunodot assay was performed by the method of Hawkes et al. (21). For identification of hybridomas producing Ab2, initial screening of the hybridomas in 96-well plates was performed by immunodot assay using Ab1, 1E4 (isotype IgM) as antigen on nitrocellulose paper. The nitrocellulose paper was blocked with 3% gelatin and incubated overnight with the supernatants of the hybridomas. Peroxidase-labeled protein A was used as secondary antibody, and dots were developed with horseradish peroxidase developer (Bio-Rad).

Slide agglutination. Bacterial agglutinations were performed on glass slides by mixing 10 μl of a saline suspension of bacteria taken from overnight growth on GC medium base agar plates with 10 μl of a 1:50 dilution of ascitic fluids containing the monoclonal antibodies. After mixing for 2 to 3 min, the agglutination patterns were read in indirect light.

ELISA analysis. Purified capsular polysaccharides were diluted in carbonate buffer (pH 9.6) to a final concentration of 10 μg/ml. The wells of 96-well polyvinyl plates (Linbro/Titertek; Flow Laboratories, Inc., McLean, Va.) were coated with 200 μl of polysaccharide buffer and incubated for 1 h at 37°C and overnight at 4°C. Plates were washed three times with 0.01 M PBS-0.05% Tween 20. Plates were blocked for 1 h with 3% gelatin at 37°C and washed. Serial dilutions of ascitic fluid starting at 1:50 to 1:100,000 were prepared, and 200 μl was added per well. NS-1 ascitic fluid or Sp2/0-Ag14 ascitic fluid served as negative control, and a 1:100 dilution of immune mouse serum served as positive control. Plates were incubated for 1 h at 37°C and washed three times. The plates were incubated for 1 h with peroxidase conjugate, washed, and developed with 200 μl of OPD substrate (12, 42). The absorbance was read with the EIA Reader (Bio-Tek Instruments Inc.). Linear regression analyses of ELISA and ELISA inhibition were performed by the method of Torrie and Steele (44) with $P < 0.05$.

Inhibition ELISA. Inhibition ELISA was performed by the method of Apicella and Gagliardi (3). In the inhibition studies with Ab1, 80 isolates of *N. meningitidis* were tested for their ability to inhibit the binding of monoclonal antibodies to group C polysaccharides. ELISA was carried out as described above. Microdilution plates were coated with 10 μg of group C polysaccharide per ml. With the test plates described above, no need for polylysine precoating or polysaccharide-polylysine conjugation was necessary. As inhibitors, a suspension of whole organisms was prepared in PBS-0.5% gluteraldehyde (optical density at 660 nm of 0.3) and diluted 1:10. An equal volume of Ab1 produced in ascitic fluid and diluted at 1:3,000 was added to the organisms and incubated for 1 h at 37°C. The organisms were removed by centrifugation. The absorbed Ab1 was tested in ELISA. Each strain was evaluated four to eight times, and results were compared to inhibited controls (homologous organisms against which Ab1 monoclonal antibodies were made) and

uninhibited controls (unabsorbed antibody) which were included on each plate.

In the inhibition studies with Ab2, Ab1 produced in ascitic fluid and diluted 1:6,000 was incubated with excess amounts of potential Ab2 antibodies for 1 h at 37°C. The absorbed antibody was centrifuged and was tested for its ability to react with group C capsular polysaccharide in ELISA under the conditions described above. Other Ab1 inhibitors which were tested similarly at the same time included meningococcal C polysaccharide (inhibited control) and monoclonal antibody 3E4 (uninhibited control). Ab2 monoclonal antibodies were also tested for their ability to inhibit human antibody reactivity to group C capsular polysaccharide in a similar manner. To accomplish this, sera obtained from a patient convalescing from meningococcal C sepsis was incubated with various quantities (6 to 200 μg/ml) of inhibitors for 1 h at 37°C. The reaction mixture was centrifuged and was tested for reactivity in an ELISA specific for group C polysaccharide.

Bactericidal assay. The bactericidal assay used was a modification of the test described by Gold and Wyle (16). All dilutions were made in Geys balanced salt solution (GIBCO Laboratories, Grand Island, N.Y.). The source of complement used was 8% human hypogammaglobulinemic serum or rabbit serum which lacked intrinsic bactericidal activity against the test strains. *N. meningitidis* organisms were grown overnight at 37°C in Mueller-Hinton broth. The following day, 10 ml of overnight growth was resuspended in 90 ml of Mueller-Hinton broth and incubated at 37°C with shaking until organisms reached early log phase. The bacterial suspensions were diluted in Geys balanced salt solution to a concentration of 4×10^4 CFU/ml. Reaction mixtures were set up in sterile glass tubes (12 by 75 mm) and consisted of 50 μl of meningococci, 20 μl of complement source, 25 μl of antibody, and 155 μl of Geys balanced salt solution. Controls included a serum of known bactericidal activity, antibody without complement, complement source without antibody, and NS-1 ascitic fluid. The reaction mixture was incubated with shaking at 37°C. Viable colony counts were obtained at 0, 30, and 60 min by plating 25 μl of the reaction mixture on supplemented GC agar. Colonies were counted after 18 to 24 h of incubation at 37°C in 5% CO_2.

Immunofluorescence studies. Hybridoma cells which were suspected of secreting potential Ab2 were studied to determine whether epitopes that cross-react with the meningococcal C antigens could be detected on their surfaces. These hybridoma cells were washed extensively in PBS and incubated for 1 h at room temperature with anti-rabbit meningococcal C typing sera obtained from the Centers for Disease Control. These sera had been exhaustively absorbed with Sp2/0-Ag14 plasmacytoma cells to remove background heterophile antibody. A separate set of cells from these hybridomas was stained with Centers for Disease Control group A meningococcal rabbit typing sera similarly absorbed with Sp2/0-Ag14 plasmacytoma cells. After being stained with the respective polyclonal antibodies, the cells were washed thrice in PBS, and fluorescence was developed with fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (46).

Rabbit immunizations. Monoclonal anti-idiotype antibody and meningococcal C polysaccharide (Merck Sharp & Dohme) were coupled to keyhole limpet hemocyanin (KLH) with glutaraldehyde (33). Three rabbits were immunized subcutaneously with 100 μg of anti-idiotype-KLH, meningococcal C polysaccharide-KLH, and KLH suspended in Freund complete adjuvant on days 0 and 14. These rabbits received an intravenous injection of either 100 μg of anti-idiotype antibody, meningococcal C polysaccharide, or saline on day 28. In addition, two rabbits were immunized intravenously with 100 μg of either anti-idiotype antibody or meningococcal C polysaccharide without adjuvants on days 0 and 14. The rabbits were bled on day 0 and thereafter on a weekly basis, and their sera were tested for an anti-meningococcal C polysaccharide response in ELISA.

RESULTS

Development of a monoclonal antibody to the meningococcal C polysaccharide. The initial experiments leading to the development of Ab2 which mimicked the meningococcal capsular polysaccharide necessitated the development of a monoclonal antibody to this polysaccharide. Monoclonal antibody 1E4, which was an IgM isotype, was identified from the fusion of the splenocytes of the strain MP13-immunized mice. This antibody recognized several different preparations of purified group C capsular polysaccharides (MP1, MP13, and Merck Sharp & Dohme vaccine polysaccharide) in immunodot assay. Western blot procedures with *N. meningitidis* MP13 as antigen indicated that the antibody did not recognize any other cell component. The high-molecular-weight polysaccharide capsule did not migrate into sodium dodecyl sulfate-polyacrylamide gels, in contrast to outer membrane proteins and lipooligosaccharides; thus no bands were visualized on Western blots. The ascitic fluid containing 1E4 was tested in ELISA for recognition of purified capsular polysaccharide (Fig. 1). Regression analysis indicated that at the 95% confidence level, reactivity of monoclonal antibody 1E4 ascitic fluid was significantly greater than that of control to a dilution of 1:100,000. Inhibition ELISA studies indicated that 50% inhibition of 1E4 could be achieved with 10 μg of MP13 capsular C polysaccharide per ml. Meningococcal A capsular polysaccharide failed to inhibit the 1E4 ELISA at concentrations as high as 200 μg/ml.

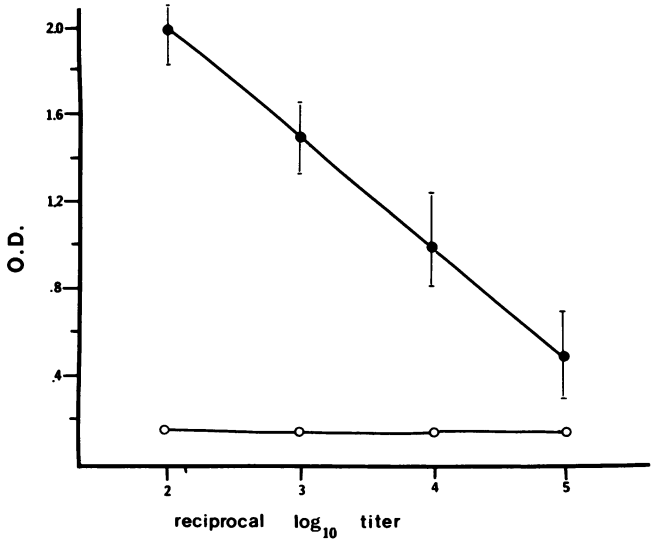

FIG. 1. ELISA analysis of monoclonal antibody 1E4 ascitic fluid (●) and Sp2/0-Ag14 ascitic fluid control (○) with meningococcal C polysaccharide. The wells were coated with 10 μg of meningococcal C polysaccharide per ml. Each curve was generated by simple regression analysis of data from eight separate measurements at each point. Vertical bars represent the 95% confidence limits at these points. O.D., Optical density.

To determine the prevalence of the antigenic determinants recognized by 1E4 among other *N. meningitidis* strains, we studied 30 group C isolates, 34 group B strains, 6 group A strains, 6 group Y strains, 2 group W135 strains, and 2 group X strains. Reactivity was evaluated by dot assay, inhibition ELISA, slide agglutination, and bactericidal assays. Optimal concentrations of the antibodies used in each assay had been determined previously by using strain MP13. Monoclonal antibody 1E4 recognized determinants on all group C strains tested and on 1 (strain 6275) of 34 *N. meningitidis* serogroup B strains tested (Table 1). The antigenic determinant recognized by 1E4 was uniformly absent on the capsules of all serogroup A, Y, W135, and X isolates examined. Studies with 1E4 in a bactericidal assay using strain MP13 demonstrate a prozone effect to a dilution of 1:800 (Fig. 2). One hundred percent killing was observed up to a dilution of 1:10,000.

The above-described studies demonstrate that monoclonal antibody 1E4 had a specificity for an epitope that was common to the capsular polysaccharide of the meningococcal group C strains studied. In addition, the epitope was a target for bactericidal antibody. These studies led to the selection of this antibody as the idiotype (Ab1) for the development of the anti-idiotype antibody (Ab2) which would have the surrogate image of the meningococcal C polysaccharide epitope as its antibody combining site.

Development of the anti-idiotype (Ab2) with a surrogate image of meningococcal C polysaccharide. Previous studies in our laboratory indicated that the sera of mice used to produce ascites to monoclonal antibodies frequently contained evidence of an anti-idiotype (Ab2) response to these antibodies. On the basis of this observation, we elected to use spleens from ascitic mice in which hybridoma 1E4 had been implanted as sources of splenocytes for the generation of these Ab2-secreting hybridomas. Initially, hybridomas from these fusions were screened for recognition of Ab1, 1E4 in immunodot assay. Six hybridomas positive in this immunodot assay were subjected to further studies.

ELISA inhibition studies. The six anti-idiotype antibodies which recognized the idiotype Ab1, 1E4 in immunodot assay were tested for the ability to inhibit 1E4 in an ELISA specific for the capsular C polysaccharide. One of these antibodies, designated 6F9, showed greater than 70% inhibition of 1E4 in this ELISA on repeated occasions. The other five antibodies failed to inhibit 1E4 in this ELISA. These studies suggested that monoclonal 6F9, an IgG2b isotype, was potentially expressing a surface epitope closely related to or immunologically identical to the meningococcal C polysaccharide. Prior to further studies, hybridoma 6F9 was cloned twice by limiting dilution.

Immunofluorescence studies. Anti-idiotype antibodies which behave as surrogate antigens should also bind polyclonal sera raised in different animal species in which it is less likely that idiotypes unrelated to antigen binding would be identical (13, 26, 46). Cells from the six anti-idiotype hybridomas were tested for the presence of surrogate antigen by the indirect immunofluorescence technique. These studies demonstrated that hybridoma 6F9 stained circumferentially with anti-C typing sera (Fig. 3A) and failed to stain with anti-A typing sera (Fig. 3B). Other hybridomas which produced antibodies which bound to 1E4 but did not inhibit 1E4 polysaccharide binding were also tested in these systems. No fluorescence was detected with these cells with either rabbit antisera. It is recognized that these polyclonal typing sera contain antibodies to other meningococcal determinants besides the capsular polysaccharides. However, the reactivity of 6F9 with 1E4, and the ability of 6F9 to inhibit 1E4 binding to meningococcal C polysaccharide in ELISA suggested that the fluorescence seen in these studies had a high probability of representing rabbit anti-C capsular antibody binding to a surface epitope on the 6F9 hybrid which was a surrogate image of the C polysaccharide.

TABLE 1. Prevalence of antigenic determinants recognized by monoclonal antibody 1E4

Serogroup	No. of strains tested	No. (%) of strains positive by indicated assay		
		Dot assay	Slide agglutination	Inhibition ELISA
C	30	30 (100)	30 (100)	30 (100)
B	34	1 (3)	1 (3)	1 (3)
A	6	0 (0)	0 (0)	0 (0)
Y	6	0 (0)	0 (0)	0 (0)
X	2	0 (0)	0 (0)	0 (0)
W135	2	0 (0)	0 (0)	0 (0)

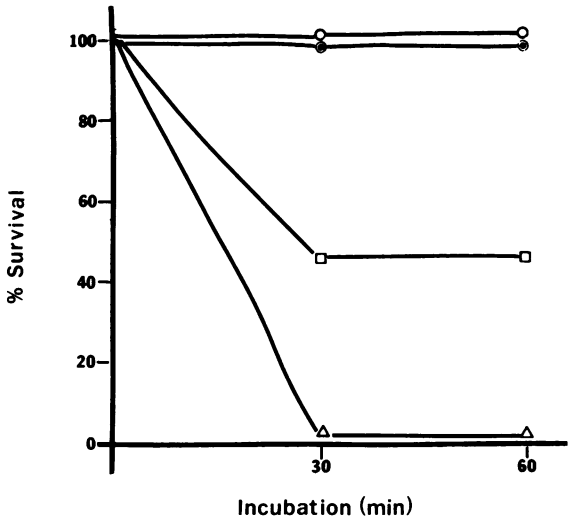

FIG. 2. Bactericidal activity of monoclonal antibody 1E4 ascitic fluid against the homologous strain, MP13. Results of a control assay utilizing SP2-0 ascites (○) is also shown. Monoclonal antibody 1E4 was diluted 1:100 (⊙), 1:1,000 (□), and 1:10,000 (△). Each experiment was carried out in triplicate on three separate occasions. The graph shows mean values of nine studies.

ELISA inhibition studies with human serum. In a series of experiments designed to determine whether 6F9 could bind to anti-capsular C antibody from another animal species, the ability of 6F9 to bind human antibodies was studied in a meningococcal C polysaccharide-specific inhibition ELISA (Fig. 4). Complete (100%) inhibition of human antibodies in this ELISA was achieved with 200 μg of 6F9 per ml, and 50% inhibition was achieved with 50 μg of 6F9 per ml. Similar degrees of inhibition were obtained with 200 and 32 μg of C capsular polysaccharide per ml, respectively. Monoclonal antibody 3E4 failed to inhibit human antibodies in this ELISA.

Rabbit immunizations with 6F9. The ability of 6F9 and meningococcal C polysaccharide to generate anti-C capsular polysaccharide immune responses in rabbits was studied. The preimmune sera of all rabbits studied did not contain anti-meningococcal C polysaccharide antibodies as measured in ELISA. Similarly, a significant anti-meningococcal C polysaccharide antibody response could not be detected

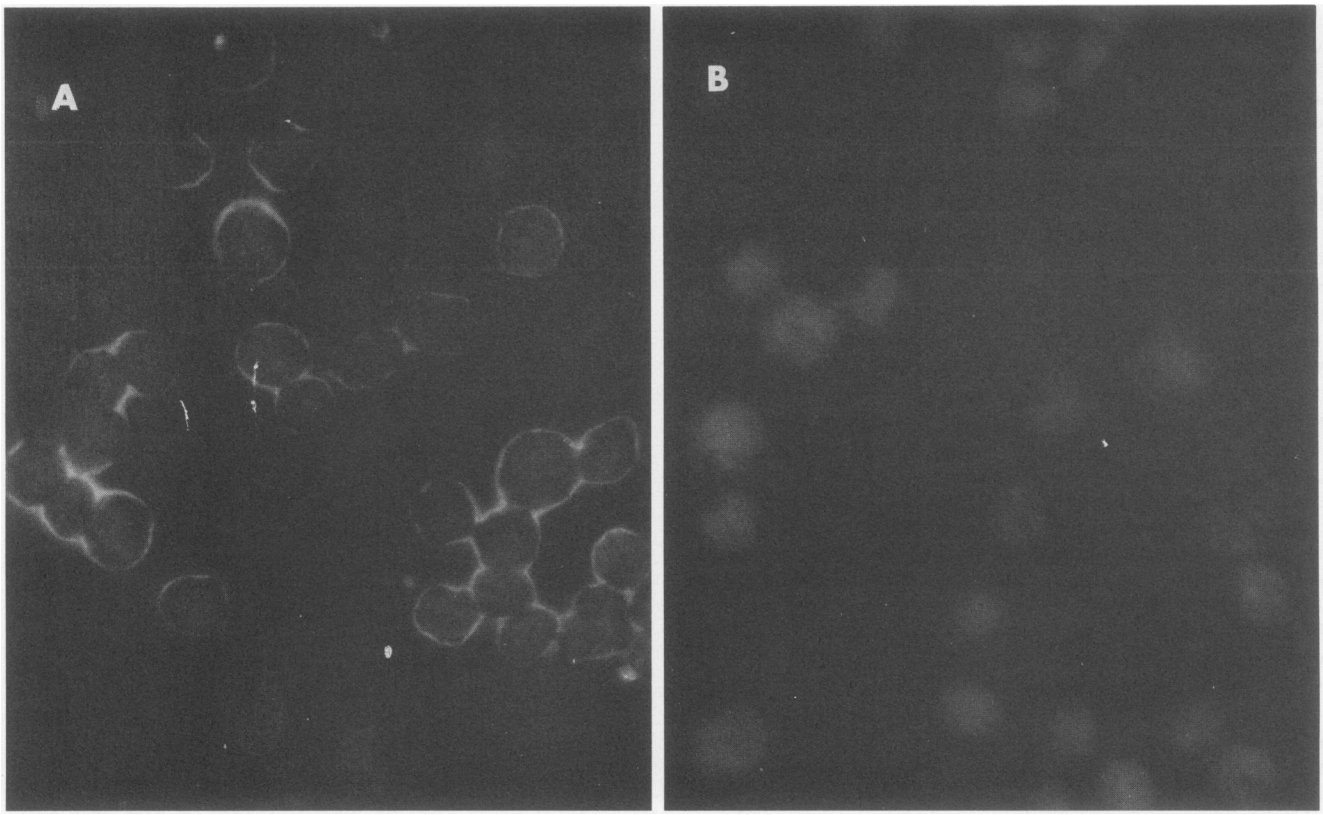

FIG. 3. Results of indirect immunofluorescence assay. (A) Circumferential binding of 6F9 cells to antibody after treatment with anti-C capsular typing sera. (B) Absence of fluorescence after staining 6F9 cells with anti-A capsular typing sera.

after primary or secondary immunization with 6F9 or meningococcal C polysaccharide. Three rabbits were then immunized with 6F9-KLH, meningococcal C polysaccharide-KLH, and KLH suspended in Freund complete adjuvant. At 2 weeks after the second immunization, an anti-meningococcal C polysaccharide IgG response was detected in the sera of rabbits injected with meningococcal C polysaccharide and 6F9 to a dilution of 1:160 (Fig. 5a). Subsequent intravenous booster injections on day 28 of these animals resulted in a significant rise in anti-meningococcal C polysaccharide antibody titer measured on day 33. The rabbit immunized with meningococcal C polysaccharide generated an anti-meningococcal C polysaccharide IgG antibody response which was significantly greater than that of the KLH control to a titer of 1:160 (Fig. 5b). The serum of the rabbit immunized with the 6F9 revealed an anti-meningococcal C polysaccharide IgG antibody response which was significantly greater than that of the KLH control to a titer of 1:50,120. Simultaneous measurements of anti-meningococcal C polysaccharide IgM responses in these sera indicated titers which were not significantly different from those of KLH controls. To demonstrate the specificity of this antibody response, the sera were tested for reactivity with meningococcal A polysaccharide in ELISA. All three rabbit antisera failed to react. These results indicate that 6F9 contained an antigenic epitope which mimicked meningococcal C capsular polysaccharide. Coupled to a protein carrier, 6F9 functioned as an effective immunogen and stimulated an anti-meningococcal C polysaccharide IgG antibody response which was significantly greater than the response obtained with a conjugated meningococcal C polysaccharide preparation in rabbits.

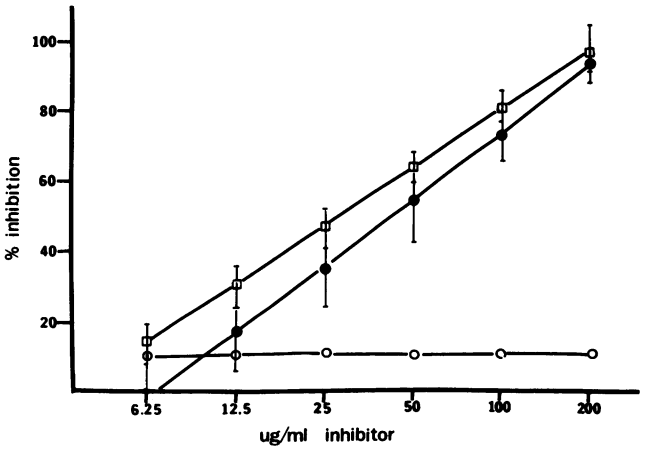

FIG. 4. Results of inhibition ELISA specific for the meningococcal C capsular polysaccharide with human hyperimmune sera. To accomplish this inhibition, human sera at a final dilution of 1:7,500 was absorbed with various concentrations of inhibitor. Microdilution wells were coated with 10 μg of C polysaccharide per ml. Symbols: (●), 6F9; (□), meningococcal C polysaccharide; (○), 3E4. The inhibition curves were generated by regression analysis of data from separate analysis at each point. The vertical bar represents the 95% confidence limits at these points.

Monoclonal antibody Ab3. The functional capacity of 6F9 to act as a surrogate image of meningococcal C polysaccharide was exploited in the production of monoclonal antibody

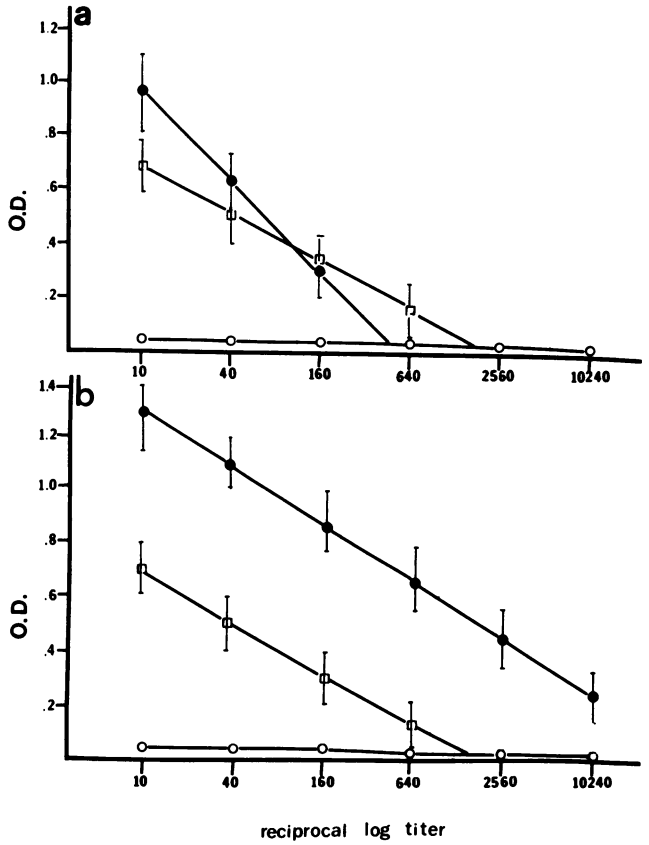

FIG. 5. ELISA analysis of rabbit sera with meningococcal C polysaccharide. (a) Rabbits were immunized subcutaneously with 100 μg of 6F9-KLH (●), 100 μg of meningococcal C polysaccharide-KLH (□), or 100 μg of KLH (○) emulsified in Freund complete adjuvant on days 0 and 14. The anti-meningococcal C polysaccharide IgG response was measured on day 27. Microdilution wells were coated with 10 μg of C polysaccharide per ml. Each curve was generated by regression analysis of data from eight separate measurements at each point. Vertical bars represent the 95% confidence limits at these points. (b) Rabbits were immunized as for panel a and received an intravenous booster injection of 100 μg of 6F9 (●), 100 μg of meningococcal C polysaccharide (□), or 100 μg of KLH (○) on day 28. The anti-meningococcal C polysaccharide IgG response was measured on day 33. Microdilution wells were coated with 10 μg of polysaccharide per ml. Vertical bars represent the 95% confidence limits.

Ab3. Splenocytes of BALB/c mice immunized intraperitoneally with 6F9 hybridoma cells were fused with Sp2/0-Ag14 plasmacytoma cells. Hybridoma cells were screened for recognition of meningococcal C polysaccharide in immunodot assay. Two monoclonal antibodies, 1F2 and 1G11, both of the IgM isotype, were identified which reacted with meningococcal C polysaccharide. The reactivities of these Ab3 monoclonal antibodies were tested in immunodot assay against 12 strains of group C, 6 strains of group B, and 2 strains of group A meningococci. Both Ab3 monoclonal antibodies reacted with 12 of 12 group C strains, 1 (strain 6275) of 6 group B strains, and 0 of 2 group A strains tested. The ascitic fluids containing 1F2 and 1G11 were bactericidal (100% inhibition of growth) at dilutions from 1:500 to greater than 1:100,000 for the two strains of group C organisms tested. The Ab3 monoclonal antibodies were not bactericidal for the group A meningococcal strain tested. The reactivities of these Ab3 monoclonal antibodies were similar to that of monoclonal antibody Ab1, 1E4. The results of these experiments indicate that Ab3 monoclonal antibodies induced by immunization with 6F9 (Ab2) were bactericidal antibodies with specificities for group C capsular polysaccharide of the meningococcus identical to that of Ab1, 1E4.

DISCUSSION

The serogroup differentiation of meningococci is based on capsular polysaccharide antigens (39). Kabat and coworkers (23) showed that these antigens are important in immunity by demonstrating that rabbit and horse antisera to serogroup A meningococci lose much of their protective capacity after absorption with group A polysaccharide. Goldschneider et al. (17) observed that the presence of bactericidal activity of serum correlates with protective immunity in humans. The successful isolation and purification of a high-molecular-weight polysaccharide (19) led to the development of group A and group C polysaccharide vaccines capable of inducing group-specific, complement-dependent, bactericidal antibodies in adults (4, 19, 20). However, these T-cell-independent antigens fail to elicit a significant immune response in infants under 2 years old. A novel approach to converting T-cell-independent antigens into T-cell-dependent antigens is by utilizing so-called internal antigens or anti-idiotypes. Anti-idiotypes may be coupled to potent carriers and act as T-dependent antigens. This latter technique is based on the immune regulatory network theory of Jerne (22) that defines the immune system as a web of interacting idiotypes. Antibodies carry idiotypes, which are regions in or near the antigen recognition sites. Idiotypes are capable of acting as antigens and of stimulating antibody production. The normal immune system features an interlocking network of antibodies directed at one another's idiotypes. Production of any particular antibody will be suppressed by its corresponding anti-idiotype antibody. Inherent in the network theory is the idea that at least some of the anti-idiotype antibodies are internal images of external antigens. That is to say that certain anti-idiotype antibodies express structures which resemble the structure of external antigens. For an antigen and an anti-idiotype antibody to bind to the same region of an antibody, they must have similar three-dimensional configurations, even though they may be different biochemically.

Nisonoff and Lamoyi and Roitt and co-workers (36, 40) suggested in 1981 that internal image antibodies might be exploited as a new type of vaccine. By using monoclonal antibody technology, protective antibodies to antigenic material are produced. These protective antibodies are subsequently purified and used to produce anti-idiotype antibodies which will be the image of the original antigen. When used as a vaccine, the anti-idiotype antibody will elicit protective immunity to the original pathogen. Several authors have employed this technique successfully and raised protective antibodies in animals against various pathogens, including *Escherichia coli* K13 (45), hepatitis B (25), *S. pneumoniae* (32), and a number of parasitic and viral systems (28). Idiotype vaccines offer several distinct advantages over conventional vaccines. Idiotype-based vaccines do not contain nominal antigen nor its fragments; thus these vaccines do not have the same undesired side effects which may be associated with conventional vaccines. Idiotype vaccines do not depend on the availability of large amounts of pure antigen, which is often a limiting economic factor in vaccine production. Finally, idiotype vaccines are proteins and can be easily manipulated and coupled to potent immunogenic

carriers to become T-cell-dependent antigens which can receive full T-cell help (28). Ideally, an anti-idiotype vaccine should mimic a highly conserved epitope and offer protection against all strains of one serogroup.

In this report, we describe the monoclonal idiotypic antibody (Ab1) which was selected to produce the anti-idiotypic antibody (Ab2). Ab1 recognized a conserved epitope which was a target for bactericidal antibodies present on the capsular polysaccharides of 30 of 30 strains of *N. meningitidis* group C tested. This syngeneic monoclonal idiotype was used for the production of monoclonal anti-idiotypes. Not all anti-idiotypes are so-called internal images. Anti-idiotypes are classified by their physical relation to the antigen binding site (29). Alpha anti-idiotypic antibodies (Ab2 alpha) are directed against idiotypes on the variable antibody domain which are not near the antigen binding site. Anti-idiotypic antibodies which can induce steric interference with antigen binding, i.e., which are directed against an idiotype near the binding site, are called Ab2 gamma. Both Ab2 alpha and Ab2 gamma are subject to genetic restriction. Anti-idiotypes which serve as surrogates of the nominal antigen are capable of competing with the nominal antigen for the binding site and are called Ab2 beta (8). The binding of Ab2 beta to Ab1 mimics the binding of antibody to antigen and is not genetically restricted. It is therefore important to correctly identify Ab2 beta. In initial screening of the anti-idiotype hybridomas, a total of six hybridomas were identified which recognized the idiotypic antibody (Ab1). Only one of these anti-idiotypes, monoclonal antibody 6F9, inhibited the binding site of the nominal antigen in inhibition ELISA.

These results suggest that 6F9 recognized an idiotypic determinant very close to, if not identical with, the paratopic regions of the combining site. Two assays were designed to test whether the specificity of 6F9 was directed at a region near the 1E4 binding site (Ab2 gamma) or at the antigen binding site (Ab2 beta). For this purpose, polyclonal, anti-meningococcal C polysaccharide serum raised in a different species in which it was unlikely that idiotypes unrelated to antigen binding would be identical was used. Monoclonal anti-idiotype 6F9 inhibited the interaction of human anti-meningococcal C polysaccharide antibodies with antigen in solid-phase assay.

An indirect immunofluorescence technique which was previously demonstrated to be useful in identifying appropriate anti-idiotype monoclonal antibodies in a hepatitis B antigen model (46) was also used. Monoclonal antibody 6F9 showed circumferential staining with rabbit anti-meningococcal C polysaccharide typing sera and failed to stain with rabbit antimeningococcal A polysaccharide serum. Furthermore, 6F9-KLH conjugate evoked an IgG antibody response specific for the meningococcal C polysaccharide in rabbits. These date indicate that internal-image-bearing anti-idiotype do not appear to be genetically restricted. Few other studies (13, 26) have demonstrated the induction of Ab3 by using monoclonal anti-idiotype across species barriers. Finally, immunization of mice with 6F9 hybridoma cells resulted in the production of monoclonal antibody Ab3 which has the same specificity as Ab1, thus completing the network theory of Jerne (22). These data confirm the fact that 6F9 is an Ab2 beta and functions as a surrogate antigen.

In conclusion, we have used a bactericidal monoclonal antibody which recognized the capsular polysaccharides of 30 of 30 strains of *N. meningitidis* group C tested to produce an anti-idiotype antibody which was the surrogate or so-called internal image of capsular polysaccharide. This anti-idiotypic antibody will allow us to study the B- and T-cell responses to the protein image of a T-cell-independent antigen in neonatal and adult animals.

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