INFECTION AND IMMUNITY, Nov. 1985, p. 510–516
0019-9567/85/110510–07$02.00/0

Vol. 50, No. 2

Protection against Infection with *Neisseria meningitidis* Group B Serotype 2b by Passive Immunization with Serotype-Specific Monoclonal Antibody

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Received 19 April 1985/Accepted 15 July 1985

Hybridomas derived from mice immunized with *Neisseria meningitidis* serogroup B serotype 2b (B,2b) outer membrane preparations produced monoclonal antibodies (MAbs) specific for major outer membrane proteins of classes 1, 2, and 5. The MAbs were examined by enzyme-linked immunosorbent assay against a selected panel of seven strains of *N. meningitidis* (B,2b) of different sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns, a serotype 2a, and a nontypable strain. The five MAbs selected were all bactericidal and of different immunoglobulin subclasses. None of the MAbs reacted with other bacterial strains in a dot-enzyme immunoassay. The corresponding antigenic determinant for each MAb was localized on a specific outer membrane protein by immunoblotting of sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of major outer membrane proteins. MAbs M5-11 and M5-30 bound to the class 2 protein and were serotype 2b specific. MAb M2-20 bound to the class 1 protein, and MAbs M5-16 and M5-19 bound to the class 5 protein. A mouse model of infection was established whereby a local infection progressed to lethal bacteremia over 3 days, and 50% of the animals were killed with an intraperitoneal injection of 10 meningococci plus 4% mucin and 1.6% hemoglobin. The ability of the MAbs to provide passive protection against experimental infection with *N. meningitidis* (B,2b) was examined. Both serotype-specific MAbs M5-11 and M5-30 were highly protective even though they were of different immunoglobulin subclasses. The class 5-specific MAb offered no protection, while the class 1-specific MAb gave limited protection. It may therefore be possible to provide protection against serotype 2b infection by using as vaccine the class 2 serotype-specific surface-exposed outer membrane protein epitopes defined by MAb M5-11 or M5-30.

Neisseria meningitidis continues to be a major cause of bacteremia or meningitis despite the availability of a tetravalent polysaccharide vaccine against serogroups A, C, Y, and W135 (14). Serogroup B organisms are presently the cause of most endemic meningococcal disease, and unfortunately purified serogroup B polysaccharide vaccine has failed to elicit human bactericidal antibody (30). Recently, attention has been focused on the development of new vaccines with modified B polysaccharides (17, 22) and serotype protein antigens (28, 31). It is well established that outer membrane proteins (OMPs) and lipopolysaccharides are the major exposed components of the cell wall of all gram-negative bacteria. However, very little information exists regarding the virulence determinants which are responsible for the pathogenesis of *N. meningitidis*. At present, most group B serotype 2 disease is due to serotype 2b (2, 32). The protein patterns of outer membranes obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) from serotype 2b strains contain up to four major proteins. The only consistent and common protein has an apparent molecular weight of 41,500 ± 1,000 (3). The relative importance of each individual protein in eliciting bactericidal antibody as well as immunoprotection has not yet been established. It is still controversial whether such common major OMPs (MOMPs) could serve as effective vaccinogens and whether antibodies bound to these MOMPs could be protective against *N. meningitidis* infections.

Monoclonal antibodies (MAbs) developed against different OMPs of *N. meningitidis* have been used for serotyping (32). The advent of MAbs also offers the possibility of studying the biological role that each MOMP plays in bacteriolysis or passive immunoprotection experiments. We produced hybridoma cell lines secreting MAbs to different MOMPs of *N. meningitidis* B serotype 2b and studied their comparative biological and biochemical characteristics. An animal infection model with uniform and high susceptibility to meningococci was developed to evaluate the protective potential of the MAbs.

MATERIALS AND METHODS

Bacterial strains. Meningococcal strains 80165 (B,2b), 80177 (B,2b), 80238 (B,2b), 80182 (B,2b), 80143 (B,2b), and 80090 (B, nontypable) were obtained from the culture collection of the National Reference Centre for Neisseria. Strains 2996 (B,2b) and 2396 (Y,2c) were supplied by J. T. Poolman, University of Amsterdam, The Netherlands, and the serotype prototype group B strains 1 through 15 were supplied by C. E. Frasch, Office of Biologics, Center for Drugs and Biologics, Bethesda, Md. Strains were grown on blood agar plates or on gonococcus (GC) medium and were confirmed by Gram stain, oxidase test, and carbohydrate utilization patterns employing modified cysteine and Trypticase agar (BBL Microbiology Systems, Cockeysville, Md.) (4). The strains were serogrouped by bacterial slide agglutination and serotyped by agar gel double diffusion (1).

Animals. C57BL/HPB and Swiss Webster/HPB mice were obtained from colonies maintained at the Animal Resources Division. All animals weighed 18 to 20 g and were housed

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under standard conditions of temperature and relative humidity with a 12-h lighting schedule. Food and water were available ad libitum.

Outer membrane preparation. The extraction of outer membranes from the bacteria was performed as described previously (5) with slight modifications. Briefly, whole cells were suspended in lithium acetate buffer (pH 6.0) with 20 mM sodium chloride and 10 mM sodium EDTA and shaken with 6-mm beads at 300 rpm for 2 h at 45°C. Cells and debris were removed by centrifugation at 15,000 × *g* for 20 min, and the supernatant was collected and centrifuged at 40,000 × *g* for 2 h. The pellet was washed three times and then suspended in distilled water. The protein content was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Mississauga, Ontario, Canada). Each preparation was diluted to 1 mg of protein per ml and stored at −70°C in aliquots.

Immunization of mice. BALB/c mice were injected intraperitoneally and in the foot pads with 20 μg of outer membrane of strain 80177 (B,2b) or strain 80182 (B,2b) suspended in Freund complete adjuvant (GIBCO Laboratories, Grand Island, N.Y.). On day 14 the mice were reinjected with 20 μg of the same preparation suspended in Freund incomplete adjuvant. On day 28, 3 days before fusion, a booster dose of 20 μg in phosphate-buffered saline (PBS) was given intravenously.

Fusion procedure. Spleen cells from immunized mice and nonsecreting, hypoxanthine-guanine phosphoribose transferase-deficient mouse myeloma SP2/0 cells were fused in a ratio of 10:1 in a solution containing 50% (wt/vol) polyethylene glycol 1000 (J. T. Baker Chemical Co., Phillipsburg, N.J.) according to the procedure described by de St. Groth and Sheidegger (11) with modifications. Myeloma cells were cultured with 20 μM 6-thioguanidine until passage 3 before fusion. Fused cells at a concentration of 10^5 cells per ml were dispensed in 96-well tissue culture plates which already contained a feeder layer of 7×10^3 murine macrophages. Cell suspensions were grown in Dulbecco modified Eagle medium (Flow Laboratories, Mississauga, Ontario, Canada) supplemented with 20% fetal calf serum (GIBCO), 2 mM L-glutamine (Sigma Chemical Co., St. Louis, Mo.), and antibiotic-antimycotic mixture (100 U of penicillin per ml, 0.25 μg of amphotericin B [Fungizone] per ml, and 100 μg of streptomycin [GIBCO] per ml) in the presence of hypoxanthine-aminopterin-thymidine selection medium (Sigma). The first readings were done on days 5 and 6, and the medium was changed on day 7 with hypoxanthine-thymidine instead of hypoxanthine-aminopterin-thymidine. As soon as possible, usually on day 10 to 12, supernatants of wells containing growing clones were tested for MAbs directed against homologous outer membrane antigens. The antibody-producing cells were recloned by limiting dilution, and ascitic fluid was produced as described previously (7).

Enzyme-linked immunosorbent assay (ELISA) procedure. Screening of hybrid clone culture supernatants was performed as described by Brodeur et al. (6) for the measurement of antibodies to gonococcal OMPs. A volume of 100 μl of outer membrane antigens, diluted in 0.05 M carbonate buffer (pH 9.6) at a concentration of 2.5 μg of protein per ml, was dispensed in each well of a Linbro E.I.A. microtiter plate (Flow) and adsorbed for 18 h at room temperature. The microplate was washed with PBS containing 0.02% Tween 20 (Sigma), and 200 μl of 0.1% (wt/vol) bovine serum albumin (BSA; Sigma) was added to each well. After 30 min at 37°C, the BSA was discarded and the plate was washed. The test supernatants were added to each well, and the plate was incubated for 1 h at 37°C. A standard serum was used as positive control. The plate was washed thrice before the addition of 100 μl of alkaline phosphatase-conjugated goat anti-mouse immunoglobulins (Miles Laboratories, Elkhart, Ind.). After 1 h incubation at 37°C, the plate was washed, and 100 μl of a 10% diethanolamine solution (pH 9.8) containing 1 mg of *p*-nitrophenylphosphate (Sigma) per ml was added. Sixty minutes later the absorbance of the solution was determined spectrophotometrically at 410 nm with a Dynatech MR 600 microplate reader. Wells in which the absorbance was greater than 0.2 were scored as positive for the presence of antibodies directed against *N. meningitidis* group B serotype 2b outer membrane antigens.

SDS-PAGE. MOMPs were resolved by electrophoresis on SDS slab gels (1.5 mm thick) by the method of Laemmli (19) with some modifications. A 10% acrylamide (Bio-Rad) resolving gel and a 4.0% stacking gel were used. The samples were prepared by mixing one part of outer membrane (1 mg of protein per ml) with four parts of the sample buffer (62.5 mM Tris hydrochloride [pH 6.8], 1% [vol/vol] glycerol, 2% [wt/vol] SDS, 0.5% [vol/vol] 2-mercaptoethanol, 0.5% [wt/vol] bromophenol blue) and heated for 5 min at 100°C. Samples (80 μl) containing 20 to 25 μg of protein were applied to each gel lane. Electrophoresis was carried out at 20 V per gel constant voltage until the bromophenol blue tracking dye entered the separating gel, at which time the constant voltage was increased to 30 V per gel. The gels were stained with Coomassie brilliant blue dye and then destained as described by Weber and Osborn (29). The following proteins (Bethesda Research Laboratories, Gaithersburg, Md.) were used as molecular weight standards: myosin (200,000), phosphorylase *b* (92,500), BSA (68,000), ovalbumin (45,000), chymotrypsinogen (25,700), β-lactoglobulin (18,400), and cytochrome *c* (12,300).

Immunoblotting procedure. After SDS-PAGE, proteins were transferred electrophoretically from the gel to nitrocellulose paper (Bio-Rad) by the method of Towbin et al. (26). A constant potential of 40 V (5 V/cm) was applied to the gel-nitrocellulose paper sandwich for 45 min in an electroblot buffer consisting of 25 mM Tris hydrochloride, 192 mM glycine, and 20% (vol/vol) methanol at pH 8.3. The transferred proteins on the blot were stained with amido black or detected by an enzyme immunoassay. To probe for bacterial antigens, the paper was soaked in 0.02% PBS-Tween solution containing 1% casein hydrolysate for 1 h at 37°C to block nonspecific protein-binding sites and then incubated for 1 h at 37°C with mouse hyperimmune sera or MAb diluted in 0.02% PBS-Tween containing 1% casein hydrolysate. The sheet was washed thrice with PBS and incubated with peroxidase-conjugated goat anti-mouse immunoglobulins (Cappel Laboratories, Cochranville, Pa.), also diluted in 0.02% PBS-Tween containing 1% casein hydrolysate. After a 1-h incubation at 37°C and three washes, the blots were soaked in a solution of *O*-dianisidine prepared as described by Towbin et al. (26). In some experiments, the blocking buffer consisted of PBS solution containing 3% BSA, 0.5% (vol/vol) Nonidet P-40, and 0.1% (wt/vol) sodium deoxycholate to renaturate the epitope. The paper was then incubated as described above.

Determination of immunoglobulin class. The supernatants from antibody-producing cells were tested against isotype- and subclass-specific antisera (Meloy Laboratories, Springfield, Va.) by the Ouchterlony double-diffusion method.

Bactericidal assay. Fivefold dilutions of the ascites fluids were made in Hanks balanced salt solution (pH 7.2) containing 0.1% gelatin (HBSG). Guinea pig serum was used as a

source of complement. Meningococci were grown on GC medium (4) for 4 to 5 h at 36°C in 5 to 10% CO_2. A sample (250 μl) of microorganisms (approximately 5×10^3/ml) was added to each mixture of 250 μl of dilution of ascites fluid and 50 μl of complement. Controls consisted of the following: 250 μl of bacteria in 300 μl of HBSG; 250 μl of ascites fluid dilution and 50 μl of HBSG; 250 μl of HBSG and 50 μl of complement; and 250 μl of SP2/0-induced normal ascites fluid and 50 μl of complement. The mixtures were kept in a 37°C water bath for 30 min, and then 50 μl of each mixture was plated on GC medium. The plates were incubated at 36°C for 24 h in 5 to 10% CO_2. The reciprocal of the serum dilution giving 50% killing of meningococci was considered to be the bactericidal titer of the ascites fluid.

Infection. For inoculation of mice, meningococci were removed from plates after approximately 20 h of incubation and suspended in PBS. The concentration of organisms was adjusted to McFarland standard no. 3. Suspensions of bacteria were mixed with an equal volume of enhancement medium, and 1 ml of the mixture containing five times the 50% lethal dose was injected intraperitoneally per mouse. All inocula were verified by colony counts. Animals were observed daily for 3 days. Bacteremia was monitored by quantitative culture of blood at varied time intervals after infection. Groups of five mice were sacrified and sampled for each time point. A subgroup of 10 mice was kept for each group for determination of lethality.

RESULTS

Comparison of OMP profiles of *N. meningitidis* group B serotype 2b strains. To select a typical *N. meningitidis* (B,2b) strain for the production of MAbs, we first analyzed the protein composition of outer membrane preparations of several strains of *N. meningitidis* (B,2b) by SDS-PAGE. Examination of the protein patterns confirmed the variability of the class 1 (43,000 molecular weight) and class 5 (27,000 molecular weight) proteins as mentioned previously (24, 27). The class 1 protein was undetectable in strains 5 and 6 and exhibited quantitative changes in related strains (Fig. 1). The class 5 protein is subdivided into two proteins of 27,000 and 25,000 molecular weight. These are present (strains 2 and 6) or absent (strains 1 and 4) in some strains, while only one protein of either molecular weight is found in others (strains 3, 5, and 7). All the different strains of serotype 2b tested so far (Fig. 1) contained the class 2 protein (40,000 molecular weight) which constitutes the serotype-specific immunodeterminant (32). The MOMPs represented in Fig. 1 were used initially for screening MAbs because they represented the major variations in the patterns of MOMPs of serotype 2b strains.

Properties of MAbs. Based on their MOMP patterns in SDS-PAGE, strains 3 and 5 were chosen for immunization to increase the possibility of obtained MAbs directed against serotype MOMPs. However, using strain 3 as immunogen, no serotype-specific MAbs were obtained. Since strain 5 was lacking the class 1 MOMP, it was further selected to develop MAbs directed against the class 2 MOMP. Several hundred hybridomas were produced from five fusion experiments. Supernatants from the initial cultures were screened for the presence of antibody by ELISA, using homologous outer membranes as coating antigens. To identify MAbs directed against different epitopes, each positive hybrid clone supernatant was further tested against a panel of heterologous serotype 2b strains as well as a serotype 2a strains and a nontypable strain. Five hybridomas secreting high levels of MAb and showing different patterns of reactivity in ELISA were selected for further characterization and study of their protective capacities.

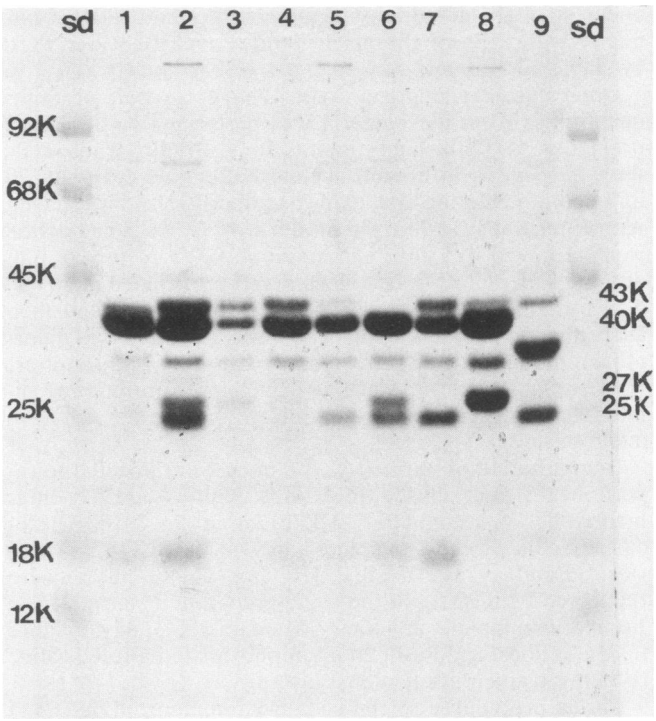

FIG. 1. Comparison of SDS-PAGE patterns of OMPs extracted from *N. meningitidis* group B serotype 2b (lanes 1 to 7), serotype 2a (lane 8), and nontypable (lane 9). sd, Standard molecular weight proteins (K, $\times 10^3$). See Table 1 for exact designation of strain numbers.

MAbs M5-11 and M5-30 reacted with all *N. meningitidis* (B,2b) strains but did not react with the prototype strains 1 through 15 including the serotype 2a and 2c strains (nontabulated) and a nontypable strain which lacks the 40,000-molecular-weight MOMP (Table 1). MAbs M5-16 and M5-19 reacted with strains possessing only the lower class 5 MOMP. These two MAbs recognized the same epitope on the nontypable strain. The M5 series of MAb was obtained with strain 5 as antigen. MAb M2-20, obtained with strain 3, demonstrated reactivity against MOMP extracted from strains having the 43,000-molecular-weight class 1 protein but did not react with strains 5 and 6 which contained no class 1 protein.

Each hybridoma was cloned twice by limiting dilution, and the class and subclass of the MAb secreted were determined by gel immunodiffusion (Table 2). The titers of these MAbs were examined by two methods, ELISA and the bactericidal test. The ELISA titer of MAb M5-19 was the highest. However, there was no correlation between ELISA titer and bactericidal titer. All MAbs were bactericidal for strain 2, which contains all the MOMPs. None of the MAbs was bactericidal for strain 8 (B,2a).

Identification of antibody-specific epitopes on the OMPs. Electroblotting followed by immunodetection was used to determine the specific OMP to which each MAb bound. The class 5 MAbs M5-16 and M5-19 generally reacted with MOMPs transferred from SDS-PAGE to nitrocellulose paper. They reacted with the lower band (25,000 molecular weight) of the class 5 MOMP, while M2-20 reacted with the 43,000-molecular-weight class 1 MOMP (Fig. 2). MAbs

TABLE 1. Reactivity of MAbs with various strains of *N. meningitidis* by ELISA

Meningococcal strains tested [a]	MOMP profile (mol wt × 10^3)			Reactivity of MAbs				
	Class 1	Class 2	Class 5	M5-11	M5-30	M5-16	M5-19	M2-20
1	42	40		+	+	−	−	−
2	43	40	27, 25	+	+	+	+	+
3	43	40	27	+	+	−	−	+
4	43	40		+	+	−	−	+
5	Tr[b]	40	25	+	+	+	+	−
6		40	27, 25	+	+	+	+	−
7	43	40	25	+	+	±[c]	±	+
8	43	40	32	−	−	−	−	−
9[d]	43		25	−	−	+	+	−

[a] The number of each strain corresponds to that shown in Fig. 1. 1, 2996 (B,2b); 2, 80165 (B,2b); 3, 80177 (B,2b); 4, 80238 (B,2b); 5, 80182 (B,2b); 6, 80171 (B,2b); 7, 81043 (B,2b); 8, M986 (B,2a); 9, 80090 (B, nontypable).
[b] Tr, Trace amount of MOMP.
[c] ELISA value of 0.13 and negative by immunoblotting.
[d] Contains class 3 MOMP (molecular weight, 35,500).

directed toward the class 2 MOMP did not react on standard immunoblots. The reactivity of the proteins was restored by treating the nitrocellulose sheets with a mixture of a nonionic detergent (Nonidet P-40) and an anionic detergent (sodium deoxycholate). These conditions restored the binding properties of MAbs M5-11 and M5-30 to their respective epitopes on the major 40,000-molecular-weight MOMP (Fig. 2). No reaction was observed with MAbs directed against *Haemophilus influenzae* antigens or normal ascites fluids.

Specificity of MAbs. A dot-enzyme immunoassay was used to rapidly screen these five MAbs against a large number of different bacterial strains. Cells from an overnight culture were suspended in saline, and 5 μl of the preparation was applied to a nitrocellulose paper and further processed as previously described for immunoblotting. MAbs M5-11 and M5-30 reacted with all strains of *N. meningitidis* (B,2b) tested. None of the five MAbs reacted with *H. influenzae* b, *H. influenzae* nontypable, *Haemophilus parainfluenzae*, *Escherichia coli*, *Streptococcus* group B, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, or *Staphylococcus epidermidis*.

Mouse infection model. To study the comparative protective capacities of the five different MAbs, we established a mouse model of infection, in which mice demonstrated uniform and high susceptibility to virulent strains of *N. meningitidis* (B,2b) by developing a progressive and fatal bacteremia. We first examined the effect of enhancement substances on bacterial infectivity in the C57BL mice (Table 3). When meningococci were suspended in 4% mucin–1.6% hemoglobin the 50% lethal dose was 10 organisms. Other vehicles used alone or in combination required a significantly larger inoculum to achieve 50% mortality. Without enhancement substances, there was 100% survival even with an inoculum of 10^9 CFU. In addition, the kinetics of bacteremia was followed by examining the blood at intervals over 2 days. Infection in C57BL mice inoculated with five times the 50% lethal dose plus mucin-hemoglobin at a concentration of 4%:1.6% could be detected as the presence of bacteria in the circulation as early as 5 h postinfection (Fig. 3). The number of organisms in the blood continued to increase to 10^9 bacteria until death occurred. Most of the animals died between 36 and 72 h postinfection, and 10 to 20% of the C57BL mice usually recovered from the bacteremia without any treatment.

Protective effect of MAbs. The protective activity of each MAb was estimated by the reduction of the level of bacteremia and the improved rate of survival. The effect of time between infection and treatment was first evaluated to determine the optimal experimental conditions for maximal protective activity.

TABLE 2. Characteristics of MAbs against *N. meningitidis* (B,2b)

MAbs	Immunoglobulin class and subclass	ELISA[a] titer	Bactericidal[b] titer
M5-11	IgG1	32,000	5,000
M5-30	IgG2a	64,000	2,000
M5-16	IgG1	8,000	6,250
M5-19	IgG2a	256,000	1,000
M2-20	IgG[c]	16,000	500

[a] ELISA titer expressed as the reciprocal of highest ascites fluid dilution with an absorbance value of 0.1 with MOMP from strain 2 as coating antigen.
[b] Reciprocal of highest dilution with strain 2. Control SP2/0-induced normal ascites fluid showed no bactericidal activity.
[c] Subclass unknown.

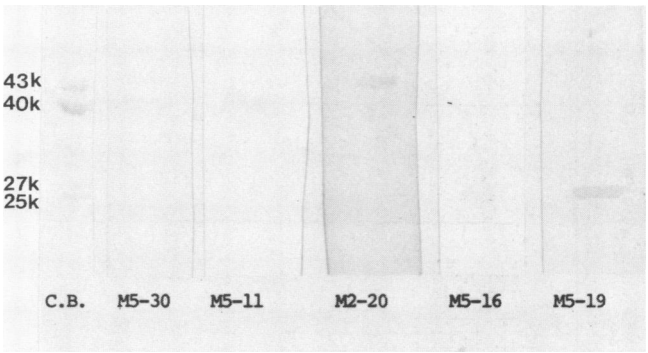

FIG. 2. Detection of MAb directed against different OMPs of *N. meningitidis* (B,2b) by immunoblotting. OMPs from strain 80165 were separated on SDS-PAGE. After electrophoretic transfer, the M5-11 and M5-30 blots were treated with PBS containing 3% BSA, 0.5% Nonidet P-40, and 0.1% sodium deoxycholate for 1 h at 37°C. The M5-16, M5-19, and M2-20 blots were soaked in 0.02% PBS-Tween containing 1% casein hydrolysate for 1 h at 37°C. C.B, Control blot. Stained with 0.1% amido black in 45% methanol–10% acetic acid and destained with 90% methanol–2% acetic acid. SP2/0-induced ascites fluid did not show any reaction (not shown). Numbers on left show molecular weights. K, ×10^3.

TABLE 3. Effect of enhancement factors on virulence[a]

Inoculum[b]	*N. meningitidis* (B,2b)	
	Concn (g/liter)	LD_{50} (CFU)[c]
Mucin	40	5×10^5
Hemoglobin	16	10^6
Iron dextran	5	5×10^4
Mucin-hemoglobin	40/16	10^1
Mucin-iron dextran	40/5	10^4

[a] No enhancement; 100% survival at 10^9 CFU.
[b] Bacteria (strain 2) plus enhancement factor (1:1) injected intraperitoneally, 1 ml per mouse, 10 mice per group. To sterilize the mucin and hemoglobin preparations, a dose of 1.5 megarads was used.
[c] LD_{50}, 50% lethal dose.

The most effective time of treatment was the injection of MAbs at 5 h postinfection (Table 4). With this schedule and when MAb M5-11 was injected intravenously, the level of bacteria in the blood after 24 h of infection was the lowest and the percent survival after 72 h postinfection was optimum. Survivors at 72 h did not succumb during a further 2 weeks of observation. The kinetics of bacteremia in mice was also monitored in groups of five mice sacrificed at 0, 5, 10, 24, 30, and 48 h postinfection. Mice in each group received 400 μl of M5-11 ascites fluid intravenously, and controls received SP2/0-induced ascites fluid. At 5 h postinfection, just before treatment, the number of bacteria in the blood was between 750 and 1,000 CFU/ml. At 10 h postinfection, a noticeable difference between the MAb-treated and mock-treated groups was apparent (Fig. 3). Between 10 and 30 h postinfection, the number of bacteria did not increase more than 10-fold in the treated group, while the level of bacteremia reached approximately 10^9 CFU/ml in the controls. After 25 h posttreatment in the treated group, the bacteria disappeared rapidly from the blood, whereas control mice died between 36 and 72 h postinfection.

Comparison of protective potential of MAbs against specific MOMPs. Class 2 and 5 OMP-specific MAbs were used in the mouse model to assess their protective capacity. It was interesting to note that both MAbs directed against the class 5 MOMP did not offer any protection against *N. meningitidis* (B,2b) strain 2 (Table 5). The class 1-specific MAb offered limited protection against the same strain. MAbs M5-16, M5-19, and M2-20 did not significantly reduce the number of bacteria in the blood after 24 h of treatment. However, both serotype-specific MAbs M5-11 and M5-30 protected 60 and 80% of the animals, respectively. The level of bacteremia was greatly reduced after 24 h of treatment when compared with that in the mock-treated mice.

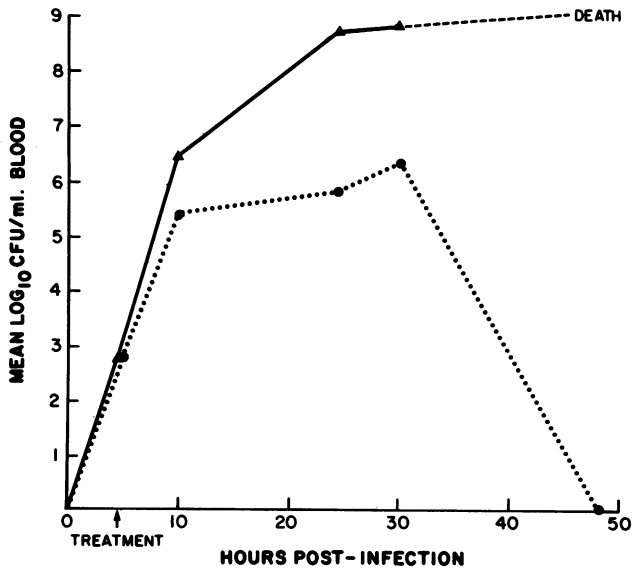

FIG. 3. Kinetics of bacteremia in C57BL mice treated with serotype-specific MAb after inoculation of *N. meningitidis* (B,2b). Symbols: ▲, control mice; ●, treated mice.

TABLE 4. Effect of the time of treatment with MAb on bacteremia and survival of mice infected with *N. meningitidis* (B,2b)

Time interval (h) between infection[a] and treatment[b]	Mean CFU/ml of blood[c]	% Survival[d]
Before infection (−0.5)	1.8×10^7	ND[e]
Postinfection		
+2	4×10^6	80
+4	3×10^7	80
+5	8×10^2	90
+6	5×10^5	50
+7	1×10^6	20
Mock treated[f]	5×10^8	20

[a] 50 organisms (strain 2) plus mucin-hemoglobin (40:16 g/liter) 1 ml per mouse intraperitoneally.
[b] 400 μl of ascites fluid (M5–11) per mouse intravenously containing 2 mg of total antibody. The ascites fluid was precipitated overnight with 40% ammonium sulfate and dialyzed at 4°C in PBS, and the protein content was determined by the Bio-Rad protein assay.
[c] 24 h postinfection, five mice per group.
[d] 72 h postinfection, 10 mice per group.
[e] ND, Not done.
[f] Mice received 400 μl of SP2/0-induced normal ascites fluid.

DISCUSSION

Several MAbs directed against the major MOMPs of *N. meningitidis* (B,2b) were prepared, and their protective activities against experimental infection in mice were evaluated as a function of antigenic specificity, immunoglobulin isotypes, ELISA, and bactericidal titers. Analysis of the antigenic characteristics of the OMPs and the recognition of common determinants as well as serotype-specific epitopes might eventually contribute to an understanding of the factors that distinguish pathogenic and virulent organisms from closely related commensal bacteria. Examination of the

TABLE 5. Immunoprotective potential of various MAbs against *N. meningitidis* (B,2b)

MAb[a]	Mean CFU/ml of blood[b]	% Survival[c]
Class 2 (M5-11)	7×10^5	60
Class 2 (M5-30)	8.8×10^4	80
Class 5 (M5-19)	1.3×10^7	20
Class 5 (M5-16)	5×10^7	0
Class 1 (M2-20)	2×10^7	40
Mock treated[d]	10^9	15

[a] 400 μl of ascitic fluid per mouse intravenously 5 h postinfection, containing 2 mg of total antibody.
[b] 24 h postinfection, five mice per group.
[c] 72 h postinfection (10 mice per group treated, 20 mice per group mock treated). 50 organisms (strain 2) plus mucin-hemoglobin (40:16 g/liter); 1 ml per mouse intraperitoneally.
[d] Mice received 400 μl of SP2/0-induced normal ascites fluid.

MOMP patterns by SDS-PAGE confirmed the variability of the class 1 (43,000 molecular weight) and class 5 (27,000 molecular weight) MOMPs of *N. meningitidis* (B,2b) strains as previously reported (24, 27). To understand and define the role of these particular surface components in infection, we developed MAbs recognizing specific epitopes on the class 1, 2, and 5 MOMPs. The five MAbs were of the immunoglobulin G (IgG) class and showed different ELISA titers. There was no correlation between the level of antibody as estimated by ELISA and the bactericidal titers. The MAbs directed towards epitopes localized on the class 1 (M2-20) or class 5 (M5-16, M5-19) OMPs could be detected easily by immunoblotting of SDS-PAGE patterns of the denatured proteins. However, as also reported by Zollinger et al. (32), the determination of the particular MOMPs to which class 2 MAbs bind was complicated by the loss of antigenic reactivity of the MOMPs after SDS treatment. The use of a dipolarionic detergent, Empigen BB, which has been found to partially restore the antibody-binding capacity of electroblotted class 2 MOMP (20), did not improve the binding detection. The native antigenic structure was restored when a mixture of the detergents Nonidet P-40 and sodium deoxycholate was used. Talbot et al. (25) reported the use of these detergents to achieve the renaturation of viral antigens. MAbs M5-11 and M5-30 with specificity for 2b MOMPs as confirmed by ELISA bound only to the class 2 principal OMP as determined by Western blotting and were, therefore, considered to have true serotype specificity. Zollinger et al. (32) have reported similar findings.

To evaluate the protective capacity of each MAb against infection with *N. meningitidis* (B,2b), we developed an animal model of infection that demonstrated high susceptibility and reproducibility. Animal models of infection reported so far often required large numbers of viable organisms and resulted in transient bacteremia and poor lethality (9, 10, 15, 16, 21). When 4% mucin and 1.6% hemoglobin were mixed with 10 CFU of meningococci and injected intraperitoneally in C57BL mice, a local infection was established which progressed to lethal bacteremia within 72 h in 50% of the animals. It has been well documented that serum bactericidal activity is a major defense mechanism against meningococci and that protection against invasion by the bacteria correlates with the presence in the serum of antimeningococcal antibodies (12, 13). In addition, passive transfer of *N. meningitidis* antiserum has been shown to be protective in animals (8, 23). Even if the MAbs are bactericidal, due to their monoclonality, there may not be a direct relationship to their ability to protect in vivo. Using a serotype-specific MAb, M5-11, we first established that the most effective time of treatment was 5 h postinfection. This time interval corresponds to the appearance of bacteria in the blood. Even though treatment of mice 2 h and 4 h postinfection provided a significant degree of protection, treatment with MAb 5 h postinfection was selected because it consistently gave a greater reduction of level of bacteremia after 24 h and a better survival rate. All the MAbs tested had varied bactericidal activity regardless of their immunoglobulin isotypes. However, their bactericidal titers as well as their ELISA titers did not correlate with the degree of protection as estimated by the level of bacteremia and percent survival. Passive immunization of mice with MAbs M5-11 and M5-30, both class 2 OMP-specific antibodies, provided protection against experimental *N. meningitidis* (B,2b) infection. In contrast to bactericidal studies, no protection was afforded by class 5 OMP-specific MAbs M5-16 and M5-19. The reason for this difference is unknown but may be attributed to an antigenic drift mechanism (24) which may occur in vivo in the presence of antibody. The ability of the class 2 OMP-specific antibodies to protect against bacteremia is not surprising considering the fact that these antibodies are directed against the serotype-specific OMP, which is present in all strains of *N. meningitidis* (B,2b) in greater amounts than the other OMPs. There was no significant difference between the protection offered by M5-11, which represents a single subclass of antibody IgG1, and M5-30, an IgG2a, when several experiments were compared. Since the level of bacteremia is directly related to the occurrence of meningitis in animals infected with *H. influenzae* b (18), any significant reduction in the number of *N. meningitidis* organisms in the circulation as a result of passive immunotherapy may prevent damage to the central nervous system.

We believe that the protection obtained in passive immunization with MAbs M5-11 and M5-30, coupled with the fact that these antibodies correspond to antibody-accessible antigenic epitopes on the class 2 serotype-specific MOMP, support further investigation of the class 2 protein as a potential vaccine candidate against meningitis due to serotype 2b meningococci.

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