Human Immunoglobulin M Paraproteins Cross-Reactive with *Neisseria meningitidis* Group B Polysaccharide and Fetal Brain

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Three hundred fifty-nine serum samples from patients with immunoglobulin M (IgM) or IgG monoclonal gammopathies were tested for binding to the capsular polysaccharide (PS) of *Neisseria meningitidis* group B (MenB PS, poly- α [2 $\rightarrow$ 8]-N-acetylneuraminic acid). Of 159 IgM paraproteins, 7 (4.4%) were positive, compared with 0 of 200 IgG paraproteins ($P < 0.05$). Since MenB PS reactivity was limited to the IgM paraproteins, the 159 IgM paraproteins were tested by enzyme-linked immunosorbent assay (ELISA) for reactivity with seven other bacterial PSs. None reacted with meningococcal A or C, *Haemophilus influenzae* type b, or *Streptococcus pneumoniae* type 3, 6, 14, or 23 PS. The specificity of the MenB PS-reactive antibodies was confirmed by demonstration of binding to *N. meningitidis* group B cells but not to a capsular PS-deficient mutant and by specific inhibition of binding to solid-phase MenB PS by soluble MenB PS in an ELISA. Five of five antibodies tested protected infant rats from bacteremia caused by *Escherichia coli* K1, an organism with a PS capsule that also is composed of poly- α [2 $\rightarrow$ 8]-N-acetylneuraminic acid. Each of the seven MenB PS-reactive paraproteins had autoantibody activity as defined by binding to homogenates of calf brain in a radioimmunoassay. For six of the seven antibodies, binding to calf brain was inhibited by the addition of soluble MenB PS. Thus, approximately 4% of human IgM paraproteins have autoantibody activity to poly- α [2 $\rightarrow$ 8]-N-acetylneuraminic acid, an antigen expressed in fetal brain and cross-reactive with the MenB capsular PS. The reason for this skewing of the IgM paraprotein repertoire toward reactivity with poly- α [2 $\rightarrow$ 8]-N-acetylneuraminic acid antigenic determinants is unknown.

Monoclonal immunoglobulins produced in multiple myeloma, Waldenström's macroglobulinemia, or other related plasma cell dyscrasias are structurally normal antibodies that can bind specifically to epitopes on proteins, carbohydrates, or nucleic acids (26). Several laboratories have investigated the reactivity of human paraproteins with polysaccharide (PS) antigens (6, 10, 19). Binding to bacterial PSs is reported to be infrequent ($\leq 2\%$ of antibodies) (10, 15, 19, 40). In contrast, autoantibody activity to PSs expressed on host tissue appears to be much more common. Up to 20% of immunoglobulin M (IgM) paraproteins are reported to react with PS antigens present on erythrocytes (7, 36). In other studies, 7 to 30% of IgM paraproteins are reported to react with PS antigens expressed on myelin-associated glycoprotein (30, 33). The epitope recognized by myelin-associated glycoprotein-reactive paraproteins is also present on sulfated glucuronic acid glycolipids (3) and mono- or disialo-ganglioside molecules expressed by neural tissues (16).

Neisseria meningitidis group B and *Escherichia coli* K1 are important causes of bacterial sepsis and meningitis (32, 35, 37, 43). The capsular polysaccharides of both organisms are structurally identical to each other, consisting of poly- α [2 $\rightarrow$ 8]-N-acetylneuraminic acid (21). Meningococcal B (MenB) PS and *E. coli* K1 PS also exhibit cross-reactivity with host polysialic acid epitopes, especially in the developing brain (11), where the cross-reactive polysialic acids are abundantly expressed on the neural cell adhesion molecule (42).

Serum antibody to MenB PS and *E. coli* K1 PS confers protection against disease caused by both organisms (13, 14,

38, 39). These PS antigens (referred to here as MenB PS) currently are candidates for inclusion in vaccines for human use. However, because of cross-reactivity with host tissues, important safety issues need to be resolved before vaccine development can proceed with these antigens. To address some of the safety issues, it would be helpful to have a source of large quantities of purified human MenB PS-reactive antibodies. However, there are only a few human MenB PS-reactive monoclonal antibodies (MAbs) available for study (20, 38, 39), and because of normally low concentrations of anti-MenB PS antibodies in human sera, it is impractical to use human serum as a source to purify large quantities of anti-MenB PS antibodies.

Kabat et al. described a human IgM paraprotein (NOV) reactive with MenB PS, which protected animals from lethal *E. coli* K1 infection (20). The frequency of such MenB PS-cross-reactive antibodies among paraproteins is unknown. In light of previous reports that a high percentage of IgM paraproteins have anti-carbohydrate autoantibody activity (7, 26, 30) and the known cross-reactivity of MenB PS with polysialic acid on host tissues (11), paraproteins might be a potential source of human anti-MenB PS cross-reactive antibodies. In the present study, we tested serum samples from patients with paraproteins for reactivity to MenB PS. For comparison, we also tested the reactivity to seven other bacterial PSs that are not known to cross-react with host antigens.

MATERIALS AND METHODS

Serum samples. Three hundred fifty-nine serum samples from patients with paraproteins were obtained from the myeloma collection of coauthor H. L. Spiegelberg at the University of California, San Diego. These samples were collected over a period of 20 years from patients residing in California and adjoining states and were stored at -20°C . The patients had Waldenström's macroglobulinemia or multiple myeloma. A total of 159 samples from patients with IgM macroglobulinemia were screened at a dilution of 1:20,000 for binding

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to MenB PS and to seven control bacterial capsular PSs (see below). In addition, serum samples from 200 additional patients with IgG paraproteins (including all four IgG subclasses) were screened at a dilution of 1:2,000 for reactivity to MenB PS only.

Serology. Anti-MenB PS antibody was measured by enzyme-linked immunosorbent assay (ELISA) using biotinylated MenB PS as the antigen. Details of the preparation and characterization of the antigen and the ELISA procedure have been described elsewhere (2). In brief, microtiter plates (Immulon 2; Dynatech Laboratories, Inc., Chantilly, Va.) were incubated overnight at 4°C with 4 µg of hen egg avidin (Extr-avidin; Sigma Chemical Company, St. Louis, Mo.) per ml dissolved in phosphate-buffered saline, pH 7.20 (PBS). The following day, the plates were washed with Tris-buffered saline (TBS)–0.1% Tween 20 (Sigma) and incubated for 2 h at room temperature with 0.5 µg of biotinylated MenB PS per ml in TBS containing 1% bovine serum albumin (BSA). After the plates were washed, serum samples diluted in TBS containing 5% fetal calf serum were added and incubated for 18 h at 4°C. Bound IgM or IgG was detected by using alkaline phosphatase-conjugated mouse MAbs specific for each of these human isotypes (HP6083 and HP6043, respectively; provided by George Carlone, Molecular Biology Department, Centers for Disease Control and Prevention, Atlanta, Ga.).

A similar ELISA method was used to measure antibodies to polyadenylic acid [poly(A)], *Haemophilus influenzae* type b (Hib), meningococcal A and C, and pneumococcal type 3, 6, 14, and 23 PSs. For Hib, wells were coated with Hib oligosaccharide-human serum albumin conjugate (34) purchased from Porter Anderson (University of Rochester, Rochester, N.Y.). Meningococcal group A and C PSs (Connaught Laboratories Inc., Swiftwater, Pa.) were mixed with methylated human serum albumin as described by Carlone et al. for sensitization of wells of microtiter plates (4). For the pneumococcal ELISA, serotype 3, 6, 14, and 23 PSs (American Type Culture Collection, Rockville, Md.) were diluted to a concentration of 20 µg/ml in PBS, added to microtiter wells, and incubated overnight (27). For measurement of antibodies to poly(A), wells were coated with poly(A) (Sigma) diluted to a concentration of 20 µg/ml in PBS. For all assays, the microtiter plates and buffers were the same as those described previously for measurement of antibodies to MenB PS by ELISA (2).

The positive controls in the IgM anti-MenB and anti-Hib PS ELISAs were two human MAbs, IgMκ 5E1 (38) and IgMκ ED 8.4 (1), specific for MenB PS and Hib PS, respectively. When tested at a concentration of 0.05 µg/ml, both human MAbs give an ELISA absorbance of >1.0 after incubation with substrate for 1 h. The positive control for the IgG anti-Men B PS assays was the chimeric IgG human MAb 5E1 (38), which gives an absorbance of >1.0 when tested at a concentration of 5 µg/ml. The positive control for the IgM anti-meningococcal A and C PS assays was a 1:200 dilution of a postvaccination human reference serum pool, obtained from George Carlone, Centers for Disease Control and Prevention. For the IgM anti-pneumococcal PS antibody assays, a serum sample from an adult obtained 1 month after vaccination with polyvalent pneumococcal PS vaccine was selected because of the presence of high serum antibody responses to each of the four antigens tested (4- to 12-fold increases in antibody, comparing titers in pre- and postvaccination sera). This serum was used at dilutions between 1:100 and 1:400 (optical density [OD] of >1). IgM NOV (provided by Elvin Kabat, Columbia University, New York, N.Y.), a MenB PS-reactive paraprotein that was reported previously to cross-react with poly(A) (20), was used as a positive control for the anti-poly(A) assay.

Reactivity of the paraproteins with intact bacteria was tested by ELISA as previously described (2). Briefly, intact bacteria were suspended in PBS and were used to coat microtiter plates. The general procedure, including choice of microtiter plates and buffers, was the same as that used for the MenB PS ELISA (2). In the present study, the sera were diluted such that each of the paraproteins was tested at a concentration of 10 µg/ml. The human anti-MenB PS MAb, 5E1, was used as a positive control, and serum containing a MenB PS-nonreactive IgM paraprotein, BEW, was used as a negative control.

Immunofixation. An immunofixation kit (Titan gel; Helena Laboratories, Beaumont, Tex.) was used to confirm the monoclonal nature of anti-MenB PS antibodies. Replicates of control and test sera were loaded on the gel and electrophoresed at 120 V for 20 min in a chamber (Titan gel chamber; Helena Laboratories). Subsequently, one set of template slots received fixative solution (30% methanol, 5% trichloroacetic acid, 3.5% sulfosalicylic acid), and the other two sets received either phosphorylcholine (1 mg/ml) or MenB PS (1 mg/ml). The gels were incubated overnight at 4°C and then washed and stained according to the manufacturer's instructions.

Anti-IgM capture ELISA. The IgM concentrations of the samples were measured by a capture ELISA using goat anti-human immunoglobulin (CALTAG, San Francisco, Calif.) as the capture antibody on the plate (2, 5). Bound human IgM was detected with alkaline phosphatase-conjugated murine anti-human IgM MAb HP6083. Quantification of IgM was based on comparison with a standard serum pool (Behringwerke Ag, Marburg Lahn, Germany) containing known concentrations of serum immunoglobulins.

Id expression. A capture ELISA was used to examine reactivity of the human paraproteins with murine anti-idiotypic (Id) MAbs (anti-Ids) produced against MenB PS-reactive human antibodies. Wells of Immulon 2 microtiter plates were coated with the following murine MAb anti-Ids: LaZ1, LaZ2, and Ra3, specific for 5E1-associated Ids (2); NOV1, NOV2, and NOV3, specific for IgM NOV-associated Ids; and Lip1, specific for IgM LIP-associated Ids (100 µl per well; 1

µg/ml in PBS). Control wells were coated with affinity-purified goat anti-human immunoglobulins (CALTAG). After washing and blocking, wells received 100 µl of approximately 1 µg of each MenB PS-reactive antibody per ml. Paraprotein BEW, which does not react with MenB PS, was used as a negative control. After overnight incubation at 4°C, wells were washed and bound human IgM was detected with alkaline phosphatase-conjugated murine anti-human IgM MAb HP6083.

Binding of paraproteins to calf brain. Brain tissue from 1-week-old calves was weighed, homogenized (Virtis, Gardiner, N.Y.) and resuspended to a concentration of 200 mg/ml in PBS. The brain homogenate was aliquoted and stored at -70°C until used. Test and control sera containing IgM paraproteins were diluted to 20 µg of IgM per ml in PBS containing 1% BSA (PBS-BSA). Serial dilutions of each of the sera (100 µl) were added to microtiter tubes containing 50 µl of the brain homogenate. After incubation at 4°C for 4 h, the tubes were centrifuged at 15,000 rpm in a microcentrifuge (Eppendorf model 5415C) for 30 s. The pellet was then resuspended and washed three times with cold PBS, after which 6×10^5 cpm of 125 I-labeled murine anti-human IgM MAb HP 6083 that had been iodinated by the chloramine-T method (41) was added. The tubes were incubated at 4°C for 2 h and centrifuged, and the pellet was washed three times with cold PBS as described above. After centrifugation, bound radioactivity in the pellet was measured with a gamma counter. A control human serum containing IgM paraprotein BEW, which does not react with MenB PS, was mixed with brain tissue and resulted in approximately 8,000 to 10,000 cpm bound in the pellet, compared with 150,000 to 250,000 cpm bound with MenB PS-reactive antibodies. The addition of diluent alone resulted in the binding of $\leq 2,000$ cpm to the brain tissue. In the data presented, binding associated with diluent alone was subtracted from the counts bound by test sera containing the paraproteins. The ability of soluble MenB PS to inhibit the binding of the paraproteins to brain tissue was determined by adding different concentrations of MenB PS to the mixture of test sera and brain homogenate. Meningococcal C polysaccharide and Hib PS added in place of MenB PS failed to inhibit binding to brain tissue. The neuraminidase sensitivity of the epitopes recognized by MenB PS-reactive antibodies was assayed by incubating the brain homogenate overnight at 37°C with either diluent alone or diluent containing neuraminidase type VI (Sigma), centrifuging the tubes as described above, resuspending and washing the pellet three times with PBS, and measuring the amount of IgM paraprotein that bound to the brain tissue.

Rat protection assay. *E. coli* K1 has a PS capsule that is chemically and immunologically identical to MenB PS (21). Animal models of *E. coli* K1 infection have proven useful to investigate the function of antibodies reactive with MenB PS (22, 39). The abilities of different paraproteins to protect against *E. coli* K1 bacteremia in infant rats were tested as described by Kim et al. (22). Briefly, Sprague-Dawley pregnant rats were purchased from Simonson Laboratories (Gilroy, Calif.). In different experiments, 4- to 6-day-old pups from 8 to 12 litters were mixed and divided into test groups of 9 to 12 animals each. Antibodies were diluted in sterile PBS containing 0.5% BSA. Animals in each test group received intraperitoneally 0.1 ml of a dilution of serum containing one of the MenB PS-reactive paraproteins. Groups of control animals received intraperitoneally a comparable dilution of serum containing a MenB PS-nonreactive human IgM paraprotein or 0.1 ml of buffer alone. Immediately after administration of the antibody, the rats were challenged subcutaneously with ~ 800 CFU (~ 80 -fold the infectious dose) of *E. coli* K1 in 0.05 ml of PBS, and the animals were returned to the nursing mothers. Approximately 18 h later, the pups were anesthetized by metofane (Pitman-Moore Inc., Mundelein, Ill.) inhalation, and blood was obtained by cardiac puncture using a heparinized syringe. The blood was diluted in PBS, and 10- and 100-µl samples of each dilution were cultured onto plates containing brain heart infusion agar (Difco, Detroit, Mich.). After incubation of the plates overnight at 35°C, the number of CFU was counted.

Bacterial strains. *E. coli* K1 strain ECH16 11/11 [$\alpha(2 \rightarrow 8)$ -N-acetylneuraminic acid] capsule and its capsular PS-deficient mutant (strain ECH16c) were obtained from Howard Raff (39); *E. coli* K92 strain A40 [serotype O13;K92:H4; $\alpha(2 \rightarrow 8)$, $\alpha(2 \rightarrow 9)$ -N-acetylneuraminic acid] capsule (12, 22) was the gift of Kwang Sik Kim (University of Southern California, Los Angeles). *E. coli* K100 strain Easter (ribosyl 1,2-ribitol 5-phosphate) capsule (44) was the gift of John Robbins, National Institutes of Health, Bethesda, Md.; *N. meningitidis* group B strain NMB [CDC 8201085; $\alpha(2 \rightarrow 8)$ -N-acetylneuraminic acid] capsule and its transposon-insertional capsular PS-deficient mutant (47) were the gifts of David Stevens (Emory University, Atlanta, Ga.).

RESULTS

Reactivities of IgM paraproteins to bacterial capsular PSs. Antibody binding to MenB PS was determined by ELISA analysis of serum samples from patients with IgM or IgG paraproteins. Of 159 sera with IgM paraproteins, 7 (4.4%) were positive (OD of >2.00 at a 1:20,000 serum dilution), compared with 0 of 200 with IgG paraproteins (OD of <0.1 at 1:2,000 serum dilution; $P < 0.05$ by chi-square test, df = 2). As summarized in Table 1, the IgM concentrations of the seven sera

TABLE 1. Designations, light-chain usage, and IgM concentrations of MenB PS-reactive paraproteins

IgM	Light-chain usage	Serum IgM concn (mg/ml) ^a
BES	κ	58
KLOT	κ	42
LOG	κ	52
VAN	κ	60
HUFF	λ	44
LIP	λ	52
SLAD	λ	35

^a Measured by an anti-IgM capture ELISA, performed as described in Materials and Methods. The normal range of human serum IgM concentration is 0.45 to 1.5 mg/ml (17).

containing MenB PS-reactive paraproteins ranged from 35 to 60 mg/ml (the normal range of human serum IgM concentration is 0.45 to 1.5 mg/ml [17]). Among the seven MenB PS-reactive paraproteins, four were κ and three were λ (Table 1). Since MenB PS reactivity was limited to the IgM paraproteins, the 159 IgM paraproteins were tested by ELISA for reactivity with seven other bacterial PSs (*N. meningitidis* groups A and C, Hib, or *Streptococcus pneumoniae* serotypes 3, 6, 14, and 23 PSs). Initial screening of sera diluted to 1:20,000 resulted in 150 samples that were clearly negative with other PSs (OD of test samples, <0.2; OD of positive controls, >1.0). The remaining nine samples showed evidence of low-level binding (OD of 0.25 to 1.2). However, this low-level binding was not PS specific because antibody binding in the nine samples retested at a 1:5,000 dilution could not be inhibited by excess (25 μg/ml) soluble homologous PS (see, for example, Fig. 3).

Monoclonality of anti-MenB PS antibody. Using immunofixation, we demonstrated that the anti-MenB PS antibody in representative paraprotein-containing serum samples was monoclonal in nature. Because only small quantities of sera were available, only IgMs HUFF, LIP, and VAN were tested by this procedure. A previously unreported phosphorylcholine-reactive paraprotein, IgM GUS, was used as a negative control. The gel treated with fixative revealed bands confirming the presence of paraproteins in the four test sera (Fig. 1A). In the gel incubated with phosphorylcholine, only the antiphosphorylcholine IgM GUS was visible (Fig. 1B, lane 1). In contrast, paraprotein bands associated with MenB PS-reactive IgMs HUFF, LIP, and VAN were evident only when the corresponding portion of the gel was incubated with soluble MenB PS

FIG. 1. Representative test sera containing antibodies reactive with MenB PS (IgMs HUFF, LIP, and VAN; lanes 2, 3, and 4, respectively) or phosphorylcholine (IgM GUS; lane 1) were evaluated for monoclonality by cellulose acetate electrophoresis followed by immunofixation. The gel was divided in three parts (fixative, phosphorylcholine, and MenB PS). After electrophoresis, the paraproteins were visualized by chemical fixation or immunoprecipitation after incubation with phosphorylcholine or MenB PS.

FIG. 2. Binding of MenB PS-reactive paraproteins to bacteria of defined capsular structure. Microtiter plates were coated with intact bacterial cells. IgM paraproteins were tested at a constant concentration of 10 μg/ml. (A) Binding of test and control paraproteins and the MenB PS-reactive human MAb 5E1 to the capsular positive strain of a group B *N. meningitidis* strain (NMB) and its transposon-induced capsular PS-deficient mutant strain. The capsular PS-deficient mutant strain (M7-) reverts to capsular sufficient (M7+) when grown in the presence of *N*-acetylmannosamine (47). (B) Binding to *E. coli* K100 (ribosyl 1,2-ribitol 5-phosphate), *E. coli* K92 [α(2→8),α(2→9)-*N*-acetylneuraminic acid], and the capsular PS-positive (K1+) and capsular PS-deficient (K1-) strains of *E. coli* K1 [α(2→8)-*N*-acetylneuraminic acid].

(Fig. 1C, lanes 2 to 4). These data confirm that the respective paraproteins are specific for anti-MenB PS or antiphosphorylcholine activity.

MenB PS-reactive paraproteins bind specifically to bacteria with poly-α(2→8)-*N*-acetylneuraminic acid capsules. To confirm that the reactivity of each of the seven paraproteins was specific for MenB PS, the antibodies were tested in an ELISA for the ability to bind to intact bacteria of defined capsular PS types. The results indicate that all seven MenB PS-reactive paraproteins bound to *N. meningitidis* group B cells (Fig. 2A). All seven of the paraproteins also reacted with the transposon-induced capsular PS-deficient mutant of *N. meningitidis* group B when the organism was grown on *N*-acetylmannosamine, a supplement that induces reversion to MenB capsular PS production (47). In contrast, there was no binding to mutant bacteria that had been grown on media containing *N*-acetylglucosamine, which does not support capsular PS synthesis.

In other experiments, the MenB PS-reactive paraproteins also reacted with *E. coli* K1 cells [poly-α(2→8)-*N*-acetylneuraminic acid] (Fig. 2B). In contrast, there was no significant binding to the respective capsular PS-deficient *E. coli* K1 mutant or to *E. coli* K100 strain Easter (Fig. 2B). The *E. coli* K100 strain has a PS capsule (ribosyl 1,2-ribitol 5-phosphate) that cross-reacts with Hib PS (44) but is antigenically unrelated

FIG. 3. Dose-response binding of paraproteins to MenB PS in an ELISA. IgM concentrations were determined by an IgM capture assay (see Materials and Methods). (A) Binding of five IgM paraproteins and the previously described human anti-MenB PS MAb 5E1 (38). (B) Binding of three IgM paraproteins, SLAD, VAN, and the previously described MenB PS-reactive paraprotein IgM NOV (20), and MAb 5E1. MenB PS-nonreactive paraprotein IgM BEW tested at a concentration of 30 μ g/ml resulted in an OD of <0.2 .

to MenB PS. The MenB PS-reactive paraproteins also did not bind to *E. coli* K92, which has a repeating [$\alpha(2\rightarrow8), \alpha(2\rightarrow9)$ -N-acetylneuraminic acid] PS capsule (12) that is reported to induce antibody cross-reactive with poly- $\alpha(2\rightarrow8)$ -N-acetylneuraminic acid (8). Note that when tested at 10 μ g/ml, the amount of IgM LOG that bound to MenB or *E. coli* K1 bacteria was much lower than that of the six other MenB PS-reactive paraproteins (Fig. 2). One possible interpretation is that IgM LOG has a binding avidity lower than those of the other antibodies, an inference also suggested by results from subsequent antibody binding titration and animal protection experiments described below.

Anti-MenB PS binding of paraproteins. The binding of each of the seven paraproteins was compared in an ELISA using biotinylated MenB PS as the test antigen (Fig. 3). For this analysis, the serum samples were diluted initially to a constant IgM concentration of 30 μ g/ml, assuming that the paraproteins accounted for $>90\%$ of the total IgM concentrations. There were significant differences in the dose-response curves of

paraprotein binding to MenB PS. In the first experiment (Fig. 3A), the relative levels of binding of five paraproteins were compared with that of a positive control, human anti-MenB PS MAb 5E1, which has been described previously (38). A serum containing a MenB PS-nonreactive paraprotein, BEW, served as a negative control and resulted in a background OD of <0.1 (data not shown). Of the five MenB PS-reactive paraproteins, IgM LIP bound the best: at an IgM concentration of 0.013 μ g/ml, this paraprotein resulted in an OD of 1.0 after 1 h of incubation with the substrate. In contrast, IgM LOG required almost a 50-fold-higher antibody concentration (2.00 μ g/ml) to achieve binding equivalent to that of LIP. The relative levels of binding of the three other paraproteins, IgMs BES, HUFF, and KLOT, were intermediate to those of LIP and LOG and almost identical to each other (Fig. 3A). In a second experiment (Fig. 3B), IgM VAN required a 20-fold-lower concentration than 5E1 for equivalent binding, whereas a higher concentration of IgM SLAD was required to give binding equivalent to that of 5E1. In this assay, IgM NOV, a previously described MenB PS-reactive paraprotein (20), was slightly more active than 5E1.

Binding of anti-MenB PS paraproteins to poly(A). IgM NOV has been reported to bind to poly(A) (20). In an ELISA with poly(A) used as the test antigen, we confirmed binding of IgM NOV (OD of >2.0 when tested at 1 μ g/ml). In contrast, two previously reported human anti-MenB PS MAbs, 5E1 and 9B10 (38, 39), and the seven MenB PS-reactive paraproteins identified in the present study were negative in this assay (OD of <0.1) when tested at 10-fold-higher concentrations.

Inhibition of binding of paraproteins to MenB PS. As a further confirmation of specificity, we tested the ability of soluble MenB PS or control bacterial PSs to inhibit the binding of the paraproteins to solid-phase bound biotinylated MenB PS by ELISA. The concentration of each of the paraproteins was adjusted so that they would result in an OD of ~ 1.0 in the absence of inhibitor. Figure 4 shows representative inhibition data for five of the paraproteins. Data also are shown for the human IgM anti-MenB PS MAb, 5E1, used as a positive control. Binding to MenB PS was inhibited $>80\%$ by the addition of 1 μ g of soluble MenB PS per ml. In contrast, no inhibition was observed with the addition, at ≥ 30 μ g/ml, of soluble control capsular PS of group A or C *N. meningitidis* or Hib. Binding of IgMs SLAD and VAN also was inhibited 80 to 100% with 1 μ g of MenB PS per ml. These paraproteins were not inhibited by any other inhibitor (data not shown).

Temperature dependence of binding of paraproteins. In previous studies, the binding of anti-MenB PS antibodies to MenB PS was highly temperature dependent (25). Therefore, we tested the effects of temperature on binding activities of the anti-MenB PS paraproteins. The anti-MenB PS ELISA was performed as described in Materials and Methods except that replicate assays were performed with overnight incubation of the antibodies at 4 or 37°C. After removal of unbound antibodies, all subsequent wash and incubation steps were performed as described in Materials and Methods. For each of the paraproteins, binding to MenB PS was greater at 4°C than at 37°C (Table 2). However, the antibodies were not all affected equally by temperature: IgMs LIP and VAN were very temperature sensitive, requiring 20- to 30-fold-higher concentrations of antibodies to achieve an OD of ~ 1.0 at 37°C compared with 4°C. In contrast, IgM SLAD was the least temperature sensitive, requiring a <2 -fold-higher concentration at 37°C compared with 4°C.

Protective activities of anti-MenB PS paraproteins. An infant rat protection model was used to assess the protective abilities of five of the seven MenB PS-reactive paraproteins

FIG. 4. Abilities of different concentrations of soluble bacterial PSs to inhibit binding of IgM paraproteins to solid-phase MenB PS in an ELISA. The concentrations of the paraprotein were adjusted such that they gave an OD of ~ 1.0 in the absence of inhibitor. MenA PS, meningococcal A PS; MenC PS, meningococcal C PS; PRP, Hib PS; Colom acid, colominic acid [lower-molecular-mass (~ 20 -kDa) polymers of $\alpha(2\rightarrow 8)$ -N-acetylneuraminic acid prepared from *E. coli* K1].

TABLE 3. Passive protection of infant rats from *E. coli* K1 bacteremia by pretreatment with MenB PS-reactive paraproteins^a

IgM paraprotein	IgM dose ($\mu\text{g}/\text{rat}$)	% of rats with bacteremia
Expt 1 (9 animals/group)		
Men B PS reactive		
LIP	10	0
HUFF	10	11
KLOT	10	44
BES	10	44
LOG	10	78
Control		
BEW	10	100
Buffer	0	100
Expt 2 (12 animals/group)		
Men B PS reactive		
LIP	10	0
LOG	10	100
LOG	100	0
Control		
BEW	100	100
Buffer	0	100

^a Rats were injected intraperitoneally with 10 or 100 μg of each of the paraproteins in 100 μl of sterile PBS-0.5% BSA. Immediately thereafter, rats were challenged subcutaneously with ~ 800 CFU of *E. coli* K1. The next day, blood was withdrawn by cardiac puncture, diluted, and cultured on brain heart infusion agar.

(Table 3). In these experiments, each rat was given either PBS-BSA or 10 or 100 μg of different paraproteins. The negative control animals received either PBS-BSA ($n = 9$) or the MenB PS-nonreactive IgM paraprotein BEW ($n = 9$). All control animals developed bacteremia within 18 h after subcutaneous challenge with *E. coli* K1 (Table 3). In contrast, none of the nine animals treated with IgM LIP were bacteremic. Bacteremia was present in 11, 44, and 44% of animals treated with HUFF, BES, and KLOT, respectively. In contrast, IgM

TABLE 2. Summary of antigen binding activities, functional activities, and Id cross-reactivities of MenB PS-reactive antibodies

IgM	Light-chain isotope	Binding to MenB PS by ELISA		Binding to poly(A) by ELISA ^a	Rat protection ^b	Id expression ^c			Binding to calf brain at 4°C		
		4°C ^d	Temp dependence ^e			LaZ1	LaZ2	NOV1	Binding RIA ^f	Inhibition by soluble MenB PS ^g	Neuraminidase sensitivity ^g
VAN	κ	1	30	0	ND ^h	0	0	0	1	1	2
LIP	λ	1	20	0	1	0	0	0	1	1	1
NOV	λ	2	9.5	1	ND	0	0	1	2	2	3
5E1	κ	2	4	0	1	1	1	0	2	1	1
9B10	κ	2	2	0	1	1	1	0	2	1	1
BES	κ	3	10	0	2	0	0	0	2	1	1
HUFF	λ	3	4	0	1	0	0	0	2	1	4
KLOT	κ	3	3	0	2	0	0	0	2	1	1
SLAD	λ	4	1.8	0	ND	0	0	1	3	1	1
LOG	κ	5	Indeterminate	0	3	0	1	0	2	4	4

^a 0, OD of <0.1 at 10 $\mu\text{g}/\text{ml}$; 1, OD of >2.0 at 1 $\mu\text{g}/\text{ml}$.

^b Ranked by percentage of infant rats developing *E. coli* K1 bacteremia after pretreatment with 10 μg of each antibody per rat. 1, <20 ; 2, 21 to 50; 3, >50 . Data are from Table 3 or our unpublished observations (5E1 and 9B10).

^c LaZ1 and LaZ2 are murine anti-Ids prepared against 5E1 that recognize Ids expressed by antibodies using V κ 1 15A light chain (2). NOV1 is a previously unreported murine MAb anti-Id prepared against IgM NOV. 1, positive (OD of >2.0); 0, negative (OD of <0.2) by an anti-Id capture assay (see Materials and Methods). Four other anti-Ids, RA3, prepared against 5E1 (2), NOV2 and NOV3, prepared against IgM NOV, and Lip1, prepared against IgM LIP, did not capture any other heterologous paraproteins.

^d Antibodies were ranked for binding activity to MenB PS by comparing the amount of each antibody (micrograms per milliliter) required to give an OD of 1.0 in an anti-MenB PS ELISA performed at 4°C. 1, <0.02 ; 2, 0.021 to 0.05; 3, 0.051 to 0.49; 4, 0.5 to 2.0; 5, >2.0 .

^e Assessed by dividing the amount of antibody (micrograms per milliliter) required to give an OD of 1 at 37°C by the amount required to give an OD of 1 at 4°C. The ratio for each antibody is shown.

^f Ranked by the concentration (micrograms per milliliter) of each antibody giving $\sim 50\%$ of maximal binding in a radioimmunoassay (RIA). 1, <1.0 ; 2, 1.0 to 4.0; 3, >4.0 .

^g Ranked by the percentage inhibition of binding to calf brain of these antibodies by coinubation with MenB PS or by neuraminidase pretreatment. 1, 80 to 100; 2, 50 to 79; 3, 20 to 49; 4, <20 .

^h ND, not done.

FIG. 5. Dose-dependent binding of MenB PS-reactive paraproteins to a homogenate of calf brain as determined by radioimmunoassay. IgM concentrations were determined by an IgM capture assay (see Materials and Methods). (A) Binding of IgM NOV, a previously described human MenB PS-reactive paraprotein (20), and 5E1 and 9B10, two previously described human anti-MenB PS MAbs (38, 39). All bound in a dose-dependent manner. (B) Binding of MenB PS-reactive test paraproteins.

LOG showed no significant protection in this experiment. In a second experiment, 100 μg of IgM LOG per rat prevented *E. coli* K1 bacteremia in 100% of animals ($n = 12$), whereas at the lower dose of ~ 10 μg per rat, no protection was evident (Table 3). The requirement for a higher dose of LOG than of the other MenB PS-reactive paraproteins to achieve protection is consistent with the possible lower avidity of LOG (Fig. 3A).

Cross-reactive Id expression of IgM MenB PS-reactive paraproteins. In a previous study, murine anti-Ids were prepared against human anti-MenB PS MAb 5E1 (2). Two anti-Ids, designated LaZ1 and LaZ2, that defined overlapping subsets of human antibodies expressing the $\kappa\text{I-15A V}_L$ region were identified (2). Subsequently, we produced three additional anti-Ids against IgM NOV and one against IgM LIP. An anti-Id capture ELISA described previously (2) was used to determine whether cross-reactive Ids were expressed on the MenB PS-reactive paraproteins. Anti-5E1 Id LaZ2 captured IgM LOG, and anti-NOV Id NOV1 captured IgM SLAD (Table 2). None of the other anti-Ids recognized Ids expressed by other heterologous paraproteins. These results indicate that there was minimal Id cross-reactivity among the MenB PS-reactive paraproteins.

Reactivities of IgM paraproteins with calf brain. MenB PS expresses antigens cross-reactive with polysialic acid antigenic determinants present in fetal tissues, especially brain (11, 42). Therefore, we used a radioimmunoassay to assess binding of the MenB PS-reactive paraproteins to calf brain. Figure 5A shows the dose-response binding of two human anti-MenB PS

FIG. 6. Ability of soluble MenB PS to inhibit the binding of the paraproteins to brain tissue. A concentration of paraprotein that bound to calf brain at $\sim 50\%$ of its maximum binding in the absence of MenB PS inhibitor was used. Different concentrations of MenB PS were added as an inhibitor, and the mixture was incubated at 4°C for 4 h. MenC PS and Hib PS added as controls did not inhibit. The data in panels A and B are for the same antibodies as used for panels A and B in Fig. 5.

MAbs, 5E1 and 9B10 (38, 39), and the previously described MenB PS-reactive IgM paraprotein, NOV (20). The human MenB PS-nonreactive paraprotein BEW and polyclonal IgM purified from pooled human serum gave only background binding in the assay (data not shown). Representative data for five of the current collection of MenB PS-reactive paraproteins are shown in Fig. 5B. All of the paraproteins bound in similar dose-dependent manners to calf brain. The only exception was IgM LIP, which appeared to bind to the calf brain more avidly than the others (Fig. 5B).

Figure 6 shows that the binding of 5E1, 9B10, and the MenB PS-reactive paraproteins BES, HUFF, KLOT, and LIP to calf brain was inhibited completely by the addition of soluble MenB PS. Two other paraproteins, SLAD and VAN, also were inhibited completely by MenB PS (data not shown). In contrast, the binding of NOV (Fig. 6A) and LOG (Fig. 6B) to calf brain was inhibited poorly or not at all by MenB PS.

The effect on the binding of anti-MenB PS antibodies after pretreating the calf brain with neuraminidase also was examined in this radioimmunoassay. The epitopes in calf brain recognized by 5E1, 9B10, and IgMs BES, KLOT, LIP, SLAD, and VAN were either very sensitive ($>80\%$ inhibition) or moderately sensitive (50 to 79% inhibition) to pretreatment with neuraminidase (Table 2). However, pretreatment of calf brain homogenate with neuraminidase had less effect on the binding of NOV (20 to 49% inhibition), and it had no significant effect on the binding of HUFF or LOG ($<20\%$ inhibition; Table 2). HUFF is of particular interest since its binding to brain is inhibited completely by MenB PS (Fig. 6B).

DISCUSSION

Poly- $\alpha(2\rightarrow8)$ -*N*-acetylneuraminic acid not only is expressed on certain encapsulated bacteria but also is abundant in a number of fetal tissues (11, 42) and, to a lesser extent, in adult tissues (42). The present data indicate that the human IgM paraprotein repertoire is skewed toward reactivity with poly- $\alpha(2\rightarrow8)$ -*N*-acetylneuraminic acid. Thus, 7 of 159 serum samples from patients with IgM paraproteins reacted with poly- $\alpha(2\rightarrow8)$ -*N*-acetylneuraminic acid. In contrast, none of the 200 IgG paraproteins tested were positive. The reason for the lower frequency of IgG reactivity is unknown. Both IgM and IgG are represented among naturally occurring serum antibodies with reactivity to poly- $\alpha(2\rightarrow8)$ -*N*-acetylneuraminic acid (9, 45). However, IgM antibodies appear to be more common than IgG antibodies (8, 45), and the former are associated with much higher avidity and functional activity (38). The absence of IgG MenB PS-reactive paraproteins in our collection could reflect a true biologic restriction between the IgM heavy-chain constant region and the MenB PS-reactive paratopes used by paraproteins. Alternatively, the IgG paraproteins with MenB PS-specific paratopes may have gone undetected because of the very low avidity of the IgG dimers (38). To avoid nonspecificity in the ELISA, the IgG anti-MenB PS antibody determinations were performed on sera diluted 1:2,000, which would have detected MenB PS-reactive paraproteins at concentrations of ≥ 2 mg/ml in undiluted serum, as determined by comparison with the antigen binding of the MenB PS-reactive human IgG MAb, 5E1 (38).

In contrast to the high frequency of IgM paraproteins reactive with MenB PS, we detected no IgM paraproteins that reacted with other bacterial PSs tested, including *N. meningitidis* A or C, Hib, or *S. pneumoniae* type 3, 6, 14, or 23 PS. This absence of paraproteins reactive with these other PSs is a striking finding. The results are unlikely to be explained by failure of the serologic assays used to detect paraproteins with low-avidity binding to these latter PSs, since in each instance the assays used were highly sensitive and used appropriate antibodies as positive controls (see Materials and Methods). Also, these results are consistent with previous data indicating that reactivity of human paraproteins with bacterial PSs is infrequent (10, 19). In contrast, reactivity of human paraproteins to carbohydrate antigens expressed on host tissues is much more common (3, 6, 36). Thus, the high prevalence of MenB PS-reactive human paraproteins in our collection is likely related to the fact that these antibodies also are autoantibodies with cross-reactivity to host antigenic determinants.

By radioimmunoassay, all seven of the MenB PS-reactive paraproteins reacted with a homogenate of calf brain (Fig. 5 and 6). By immunofluorescence, these antibodies also reacted with infant rat brain (data not shown), a result indicating that the epitopes recognized by these antibodies are not specific to calves. The lack of inhibition of binding of IgM LOG to calf brain by soluble MenB PS or by pretreatment of the brain tissue by neuraminidase likely reflects broader cross-reactivity of this antibody to epitopes other than poly- $\alpha(2\rightarrow8)$ -*N*-acetylneuraminic acid. For example, IgM NOV has previously been reported to cross-react with MenB PS, DNA, and poly(A) (20).

Among our collection of MenB-PS-reactive paraproteins, there was little cross-reactive Id expression as defined by a panel of seven anti-Ids (Table 2). Thus, unlike pathologic anti-I/i blood group autoantibodies, which are predominately κ and which share an H-chain Id designated 9G4 (46), the MenB PS-reactive paraproteins appear to be heterogeneous with respect to V_L usage and to show limited Id cross-reactivity. These findings suggest that the human autoantibodies cross-reactive

with MenB PS may comprise a relatively large number of V regions compared with the anti-I/i response (46).

The development of certain B-cell tumors may depend on continued antigenic stimulation (18, 23, 49). For example, *Helicobacter pylori* gastritis is associated with primary B-cell gastric lymphoma (49), and there is evidence for the potential role for antigenic stimulation in the clonal evolution of Burkitt's lymphoma (18). The antibody activities of numerous IgM paraproteins for both host and cross-reactive environmental antigens may reflect this stimulation (7, 15, 26). An example of this self- and microbe-antigen stimulation is the identification of IgM paraproteins that are active both with *Klebsiella* outer membrane antigens and with antigens present on human cartilage glycoproteins (15). Conceivably, B cells with autoantibody activity to poly- $\alpha(2\rightarrow8)$ -*N*-acetylneuraminic acid may be activated in hosts infected with serogroup B meningococci, *E. coli* K1, or other bacteria expressing polysialic acids (9).

Another possible mechanism for the induction of B-cell tumors may be related to aging (28, 29, 48). Plasma cell dyscrasias are diseases of old age. For example, the median age of patients with multiple myeloma and Waldenström's macroglobulinemia is over 60 years (24). During aging, a decrease in the regulation of those B cells that are most likely to synthesize antibodies that cross-react with microbial and host carbohydrates might result in an increased expression of the type of paraproteins described in this study. Future studies of cross-reactive paraproteins and related B cells may help to determine the mechanisms involved in this regulation.

In summary, the repertoire of human IgM paraproteins shows an unexpected high percentage reactivity with poly- $\alpha(2\rightarrow8)$ -*N*-acetylneuraminic acid, a PS expressed on host tissues and pathogenic bacteria. Given the previously reported association between IgM paraproteins and autoantibody activity to host carbohydrate antigens (26), these MenB PS-reactive paraproteins most likely primarily represent autoantibodies. The antibodies appear to be highly heterogeneous with respect to fine antigenic specificity. Thus, human paraproteins may be a useful source of MenB PS-reactive MAbs for investigating possible relationships between fine antigenic specificity, antibody functional activity, V-region usage, Id expression, and the differences in normal and potentially pathologic anti-MenB PS antibodies.

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REFERENCES

1. Adderson, E. E., P. G. Shackelford, A. Quinn, P. M. Wilson, M. W. Cunningham, R. A. Insel, and W. L. Carroll. 1993. Restricted immunoglobulin VH usage and VDJ combinations in the human response to *Haemophilus influenzae* type b capsular polysaccharide. Nucleotide sequences of monospecific anti-*Haemophilus* antibodies and polyspecific antibodies cross-reacting with self antigens. *J. Immunol.* 151:800-809.
2. Azmi, F. H., A. H. Lucas, H. V. Raff, and D. M. Granoff. 1994. Variable region sequences and idiotype expression of a protective human IgM antibody to the capsular polysaccharide of *Neisseria meningitidis* group B. *Infect. Immun.* 62:1776-1786.
3. Burger, D., G. Perruisseau, and A. J. Steck. 1991. Anti-myelin-associated glycoprotein antibodies in patients with a monoclonal IgM gammopathy and polyneuropathy, and a simplified method for the preparation of glycolipid antigens. *J. Immunol. Methods* 140:31-36.
4. Carlone, G. M., C. E. Frasch, G. R. Siber, S. Quataert, L. L. Gheesling, S. H. Turner, B. D. Plikaytis, L. O. Hessel, W. E. DeWitt, and W. F. Bibb. 1992.

- Multicenter comparison of levels of antibody to the *Neisseria meningitidis* group A capsular polysaccharide measured by using an enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* **30**:154–159.
5. Chacko, A., and D. M. Granoff. 1991. In vitro induction of IgG1 and IgG2 secretion by B cells of children who developed invasive *Haemophilus* disease despite vaccination. *Pediatr. Res.* **30**:124–129.
 6. Christenson, M., and J. V. Dacis. 1957. Serum proteins in acquired hemolytic anemia (auto-antibody type). *Br. J. Haematol.* **3**:153–164.
 7. Crisp, D., and W. Pruzanski. 1982. B-cell neoplasms with homogeneous cold-reacting antibodies (cold agglutinins). *Am. J. Med.* **72**:915–922.
 8. Devi, S. J., J. B. Robbins, and R. Schneerson. 1991. Antibodies to poly[(2-8)- α -N-acetylneuraminic acid] and poly[(2-9)- α -N-acetylneuraminic acid] are elicited by immunization of mice with *Escherichia coli* K92 conjugates: potential vaccines for groups B and C meningococci and *E. coli* K1. *Proc. Natl. Acad. Sci. USA* **88**:7175–7179.
 9. Devi, S. J., R. Schneerson, W. Egan, W. F. Vann, J. B. Robbins, and J. Shiloach. 1991. Identity between polysaccharide antigens of *Moraxella non-liquefaciens*, group B *Neisseria meningitidis*, and *Escherichia coli* K1 (non-O acetylated). *Infect. Immun.* **59**:732–736.
 10. Emmrich, F., D. Bundle, J. van der Zee, T. Out, G. Zenke, and K. Eichmann. 1985. Two human IgM myeloma proteins with unusual specificities for streptococcal carbohydrate-associated epitopes. *Scand. J. Immunol.* **21**:119–126.
 11. Finne, J., M. Leinonen, and P. H. Makela. 1983. Antigenic similarities between brain components and bacteria causing meningitis. *Lancet* **ii**:355–357.
 12. Glode, M. P., J. B. Robbins, T. Y. Liu, E. C. Gotschlich, I. Ørskov, and F. Ørskov. 1977. Cross-antigenicity and immunogenicity between capsular polysaccharides of group C *Neisseria meningitidis* and of *Escherichia coli* K92. *J. Infect. Dis.* **135**:94.
 13. Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein. 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. *J. Exp. Med.* **129**:1307–1326.
 14. Griffiss, J. M. 1975. Bactericidal activity of meningococcal antisera: blocking by IgA of lytic antibody in human convalescent sera. *J. Immunol.* **114**:1779–1784.
 15. Hannestad, K., and K. Sletten. 1971. Multiple M component in a single individual. III: heterogeneity of M-components in two macroglobulinemia sera with antipolysaccharide activity. *J. Biol. Chem.* **246**:6982–6990.
 16. Ilyas, A. A., R. H. Quarles, T. D. MacIntosh, M. J. Dobersen, B. D. Trapp, M. C. Dalakas, and R. O. Brady. 1984. IgM in a human neuropathy related to paraproteinemia binds to a carbohydrate determinant in the myelin-associated glycoprotein and to a ganglioside. *Proc. Natl. Acad. Sci. USA* **81**:1225–1229.
 17. Isselbacher, K. J., E. Braunwald, J. D. Wilson, J. B. Martin, A. S. Fauci, and D. L. Kasper. 1994. Harrison's principles of internal medicine, p. 2489–2496. McGraw-Hill, New York.
 18. Jain, R., S. Roncella, S. Hashimoto, A. Carbone, P. Francia di Celle, R. Foa, M. Ferrarini, and N. Chiorazzi. 1994. A potential role for antigen selection in the clonal evolution of Burkitt's lymphoma. *J. Immunol.* **153**:45–52.
 19. Kabat, E. A., J. Liao, H. Bretting, E. C. Franklin, D. Geltner, B. Frangione, M. E. Koshland, J. Shyong, and E. F. Osserman. 1980. Human monoclonal macroglobulins with specificity for *Klebsiella* K polysaccharides that contain 3,4-pyruvylated-D-galactose and 4,6-pyruvylated-D-galactose. *J. Exp. Med.* **152**:979–995.
 20. Kabat, E. A., K. G. Nickerson, J. Liao, L. Grossbard, E. F. Osserman, E. Glickman, L. Chess, J. B. Robbins, R. Schneerson, and Y. H. Yang. 1986. A human monoclonal macroglobulin with specificity for α (2-8)-linked poly-N-acetyl neuraminic acid, the capsular polysaccharide of group B meningococci and *Escherichia coli* K1, which crossreacts with polynucleotides and with denatured DNA. *J. Exp. Med.* **164**:642–654.
 21. Kasper, D. L., J. L. Winkelhake, W. D. Zollinger, B. L. Brandt, and M. S. Artenstein. 1973. Immunochemical similarity between polysaccharide antigens of *Escherichia coli* O7: K1(L):NM and group B *Neisseria meningitidis*. *J. Immunol.* **110**:262–268.
 22. Kim, K. S., A. S. Cross, W. Zollinger, and J. Sadoff. 1985. Prevention and therapy of experimental *Escherichia coli* infection with monoclonal antibody. *Infect. Immun.* **50**:734–737.
 23. Konrad, R. J., L. J. Kricka, D. B. Goodman, J. Goldman, and L. E. Silberstein. 1993. Brief report: myeloma-associated paraprotein directed against the HIV-1 p24 antigen in an HIV-1-seropositive patient. *N. Engl. J. Med.* **328**:1817–1819.
 24. Kyle, R. A., and J. P. Garton. 1987. The spectrum of IgM monoclonal gammopathy in 430 cases. *Mayo Clin. Proc.* **62**:719–731.
 25. Mandrell, R. E., and W. D. Zollinger. 1982. Measurement of antibodies to meningococcal group B polysaccharide: low avidity binding and equilibrium binding constants. *J. Immunol.* **129**:2172–2178.
 26. Merlini, G., M. Farhangi, and E. F. Osserman. 1986. Monoclonal immunoglobulins with antibody activity in myeloma, macroglobulinemia and related plasma cell dyscrasias. *Semin. Oncol.* **13**:350–365.
 27. Musher, D. M., J. E. Groover, J. M. Rowland, D. A. Watson, J. B. Struwing, R. E. Baughn, and M. A. Mufson. 1993. Antibody to capsular polysaccharides of *Streptococcus pneumoniae*: prevalence, persistence, and response to re-vaccination. *Clin. Infect. Dis.* **17**:66–73.
 28. Nicoletti, C., N. C. Borghesi, X. H. Yang, D. H. Schulze, and J. Cerny. 1991. Repertoire diversity of antibody response to bacterial antigens in aged mice. II. Phosphorylcholine-antibody in young and aged mice differ in both VH/VL gene repertoire and in specificity. *J. Immunol.* **147**:2750–2755.
 29. Nicoletti, C., and J. Cerny. 1991. The repertoire diversity and magnitude of antibody responses to bacterial antigens in aged mice. I. Age-associated changes in antibody responses differ according to the mouse strain. *Cell Immunol.* **133**:72–83.
 30. Nobile-Orazio, E., P. Marmiroli, L. Baldini, G. Spagnol, S. Barbieri, M. Moggio, N. Polli, E. Polli, and G. Scarlato. 1987. Peripheral neuropathy in macroglobulinemia: incidence and antigen-specificity of M proteins. *Neurology* **37**:1506–1514.
 31. Peeters, C. C., M. A. Tenbergen, B. Haagmans, D. Evenberg, J. T. Poolman, B. J. Zegers, and G. T. Rijkers. 1991. Pneumococcal conjugate vaccines. *Immunol. Lett.* **30**:267–274.
 32. Peltola, H. 1983. Meningococcal disease still with us. *Rev. Infect. Dis.* **5**:71–90.
 33. Peter-Bosch, E., and B. E. Smith. 1993. Peripheral neuropathies associated with monoclonal proteins. *Med. Clin. North Am.* **77**:125–139.
 34. Phipps, D. C., J. West, R. Eby, M. Koster, D. V. Madore, and S. A. Quataert. 1990. An ELISA employing a *Haemophilus influenzae* type b oligosaccharide-human serum albumin conjugate correlates with the radioantigen binding assay. *J. Immunol. Methods* **135**:121–128.
 35. Pinner, R. W., B. G. Gellin, W. F. Bibb, C. N. Baker, R. Weaver, S. B. Hunter, S. H. Waterman, L. F. Mocca, C. E. Frasch, and C. V. Broome. 1991. Meningococcal disease in the United States—1986. Meningococcal Disease Study Group. *J. Infect. Dis.* **164**:368–374.
 36. Pruzanski, W., H. Jacobs, S. Saito, E. M. Donnelly, and L. C. Lui. 1987. Cryptic cold agglutinin activity of monoclonal macroglobulins. *Am. J. Hematol.* **26**:167–174.
 37. Quagliarello, V., and W. M. Scheld. 1992. Bacterial meningitis: pathogenesis, pathophysiology, and progress. *N. Engl. J. Med.* **327**:864–872.
 38. Raff, H. V., C. Bradley, W. Brady, K. Donaldson, L. Lipsich, G. Maloney, W. Shuford, M. Walls, P. Ward, E. Wolff, et al. 1991. Comparison of functional activities between IgG1 and IgM class-switched human monoclonal antibodies reactive with group B streptococci or *Escherichia coli* K1. *J. Infect. Dis.* **163**:346–354.
 39. Raff, H. V., D. Devereux, W. Shuford, B. D. Abbott, and G. Maloney. 1988. Human monoclonal antibody with protective activity for *Escherichia coli* K1 and *Neisseria meningitidis* group B infections. *J. Infect. Dis.* **157**:118–126.
 40. Riesen, W., S. Rudikoff, R. Oriol, and M. Potter. 1975. An IgM Waldenstrom with specificity against phosphorylcholine. *Biochemistry* **14**:1052–1057.
 41. Robbins, J. B., J. J. Parke, R. Schneerson, and J. K. Whisnant. 1973. Quantitative measurement of "natural" and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. *Pediatr. Res.* **7**:103–110.
 42. Rougon, G., C. Dubois, N. Buckley, J. L. Magnani, and W. Zollinger. 1986. A monoclonal antibody against meningococcus group B polysaccharides distinguishes embryonic from adult N-CAM. *J. Cell Biol.* **103**:2429–2437.
 43. Sarff, L. D., G. H. McCracken, M. S. Schiffer, M. P. Glode, J. B. Robbins, I. Ørskov, and F. Ørskov. 1975. Epidemiology of *Escherichia coli* K1 in healthy and diseased newborns. *Lancet* **i**:1099–1104.
 44. Schneerson, R., M. Bradshaw, J. K. Whisnant, R. L. Myerowitz, J. C. Parke, Jr., and J. B. Robbins. 1972. An *Escherichia coli* antigen cross-reactive with the capsular polysaccharide of *Haemophilus influenzae* type b: occurrence among known serotypes, and immunochemical and biologic properties of *E. coli* antisera toward *H. influenzae* type b. *J. Immunol.* **108**:1551–1562.
 45. Skevakis, L., C. E. Frasch, J. M. Zahradnik, and R. Dolin. 1984. Class-specific human bactericidal antibodies to capsular and noncapsular surface antigens of *Neisseria meningitidis*. *J. Infect. Dis.* **149**:387–396.
 46. Stevenson, F. K., G. J. Smith, J. North, T. J. Hamblin, and M. J. Glennie. 1989. Identification of normal B-cell counterparts of neoplastic cells which secrete cold agglutinins of anti-I and anti-i specificity. *Br. J. Haematol.* **72**:9–15.
 47. Swartley, J. S., and D. S. Stephens. 1994. Identification of a genetic locus involved in the biosynthesis of N-acetyl-D-mannosamine, a precursor of the (alpha 2→8)-linked polysialic acid capsule of serogroup B *Neisseria meningitidis*. *J. Bacteriol.* **176**:1530–1534.
 48. Tomer, Y., and Y. Shoenfeld. 1988. Ageing and autoantibodies. *Autoimmunity* **1**:141–149.
 49. Wotherspoon, A. C., C. Ortiz-Hidalgo, M. R. Falzon, and P. G. Isaacson. 1991. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. *Lancet* **338**:1175–1176.