A HUMAN MONOCLONAL MACROGLOBULIN WITH SPECIFICITY FOR $\alpha(2 \rightarrow 8)$-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

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The $\alpha(2 \rightarrow 8)$-linked *N*-acetyl neuraminic acid (NeuNAc) polymer has unique properties that distinguish it from other bacterial capsular polysaccharides (CPS).[1] It is the major virulence factor of two human pathogens, the group B meningococcus, and *Escherichia coli* K1 (1, 2). However, unlike almost all other capsular polysaccharides, this polymer, either purified or on formalin-inactivated whole bacteria, is either nonimmunogenic or elicits low levels of antibodies in a small proportion of recipients (3). Moreover, it is susceptible to rapid hydrolysis by a mammalian enzyme, neuraminidase, resulting in degradation of the antigen. The $\alpha(2 \rightarrow 8)$ glycosidic linkage of this polysaccharide is identical to that of the terminal NeuNAc residues of gangliosides and some neural glycoproteins (4, 5). The poor immunogenicity of this polysaccharide, therefore, has been explained by the structural similarity of this antigen with the host's tissues.

Individuals whose serum electrophoresis patterns showed monoclonal peaks were screened with our collection of polysaccharides (6, 7). We have found an 81-yr-old man (NOV) whose monoclonal IgM antibody showed a precipitin line by the Ouchterlony method with both group B meningococcal and *E. coli* K1 capsular polysaccharides. Quantitative precipitin curves showed specificity for $\alpha(2 \rightarrow 8)$-linked poly-*N*-acetylneuraminic acid; no precipitin reactions were obtained with $\alpha(2 \rightarrow 9)$-linked poly-*N*-acetylneuraminic acid, the CPS of the group C meningococcus, nor with the CPS of *E. coli* K92 or K100. An *O*-acetyl-

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[1] *Abbreviation used in this paper:* CPS, capsular polysaccharide.

containing *E. coli* K1 preparation showed very low reactivity. Colominic acid, also $\alpha(2 \rightarrow 8)$-linked NeuNAc (8), precipitated only slightly due to its low molecular mass.

In this report we show that (*a*) this antibody, which was present in the serum at a concentration of 23 mg/ml, is protective against *E. coli* in newborn rats, (*b*) the antibody specificity is of especial interest, in that it crossreacts with polynucleotides and denatured DNA, but has not caused any signs and symptoms of disease, and (*c*) relevant B cells derived from the patient could be infected with EBV and grown in culture.

Materials and Methods

Case Description. NOV is an 81-yr-old white male who was found, during treatment (October 1984) for anemia and rectal bleeding to have an IgM λ monoclonal gammopathy. A carcinoma of the rectosigmoid (Dukes B) was found, and resection and end-to-end anastomosis was performed. Past medical history included probable rheumatic fever and rheumatic heart disease necessitating a Bjork-Shiley aortic valve replacement in 1971, and prostatectomy in 1975 for benign prostatic hypertrophy.

Aside from a persistent low-grade anemia (hemoglobin in the range of 10–12 g/dl), there have been no signs or symptoms referable to macroglobulinemia (i.e., no lymphadenopathy or hepatosplenomegaly, no hyperviscosity or vascular abnormalities). He has no history of protracted infections, although the bowel cancer, which was ulcerated, could possibly have been a portal of entry for enteric bacteria. We have seen no signs or symptoms of SLE or any neurologic manifestations, which might reflect untoward effects of the antibody in vivo.

Sera. Serum IgM^{NOV} was available. Other human monoclonal IgM–containing sera were IgM^{FIS} (7), which reacted with chondroitin sulfates A, B, C; IgM^{WEA} (6); IgM^{MAY} (6), reacting with Klebsiella polysaccharides containing 3,4-pyruvylated galactose; IgM^{THO}; and IgM^{COL}, both with unknown specificity, served as controls in ELISA.

Polysaccharides and Polynucleotides. We used two preparations of meningococcal CPS group B, one from Dr. Carlos Moreno (Wellcome Research Laboratories, Beckenham, Kent, United Kingdom) (9). The following polysaccharides and polynucleotides were also available: meningococcal groups C and Y; *E. coli* K1, CPS strains 016 and K235 (*O*-acetyl–negative); and the *O*-acetyl–positive and *O*-acetyl–negative form variants of strain LH (10); *E. coli* K92, strains N67 and MT1389; *E. coli* K100; dextrans N-279, B-1299S, B-1355S, and N-150-N; levan B512E and P-6; phosphomannan; inulin; also blood group substances: Beach phenol insoluble P1, Tij II 20% from second 10%, Le^a substance N-1 first IO_4, hog mucin blood group A and H substance. We also used arabinogalactan; *Arianta arbustorum* galactan, *Helix pomatia* galactan; *Helix nemoralis* galactan, pneumococcal CPS types IV, VIII, XIV, and XXVII; Rhizobium TA_1; Klebsiella K7, K1, K30, and *Hemophilus influenzae* CPS types a, b, c, d, e and f; poly(dihydro U), poly(U), poly(A), poly(G), poly(C), poly(I), $poly(dAdT)_2$, $poly(dT)_4$; chondroitin sulfates A, B, and C (6, 7). Denatured calf thymus DNA (Worthington Biochemical Corp., Freehold, NJ) was prepared by boiling for 15 min and cooling rapidly.

Equine Group B Meningococcal Antiserum. The antiserum (H46) was from bleedings of a horse injected intravenously with formalin-inactivated group B meningococcal strain B-11 as described (11). The isotype of the precipitating antibody to the group B meningococcal CPS in this antiserum is IgM; the sample used for protection tests contained 1.25 mg of antibody per milliliter of serum, as determined by quantitative precipitin analysis.

Protection Tests. 5-d-old Sprague-Dawley rats (Taconic Farms, Inc., Germantown, NY) were challenged with an i.p. injection of various doses of *E. coli* 018:K1:H7 (strain C64), observed for 72 h, and the LD_{50} was determined (12–14). Antisera were assayed for their protective properties by i.p. injection of 50 μl of serum dilutions 4 h before challenge with one LD_{50} or multiples of LD_{50} doses. Tail vein blood samples of 10–20 μl were plated

at 24 h, and colonies were counted. Results list survival times only, because, of the animals that had positive blood cultures, only two survived.

Gel Diffusion. The Ouchterlony technique was used (15). Gels consisted of 13.0 ml of 1% agarose in saline containing 0.05% sodium azide, on an 8.3 × 10.2-cm glass plate. Six wells were distributed hexagonally 6 mm from a central well; 5 μl of antiserum and various polysaccharides, DNA, or polynucleotides were added. Plates were developed in a chamber containing moist filter paper.

Quantitative Precipitin Curves. To 15 μl of a 1:10 dilution of IgM^{NOV}, or 100 μl of a 1:5 dilution of H46, increasing amounts of various polysaccharides, polynucleotides, or native or denatured DNA were added in amounts of 0.2–43 μg in a total volume of 200 μl (16). After 1 h at 37°C and 1 wk in the refrigerator (with mixing twice daily), the tubes were centrifuged at 2,000 rpm for 1 h, and washed twice in the cold with 500 μl of saline. The precipitates were then digested with 25 μl of a 1:20 dilution of concentrated H_2SO_4, 15 μl of 30% H_2O_2 were added, the precipitates were redigested, then analyzed for nitrogen by the ninhydrin method (17). The serum blank and the supernatant from the tube in which maximum precipitation was found were examined by cellulose acetate electrophoresis.

ELISA for Antimeningococcal Antibody. A Corning 96-well round-bottom plate was coated with 100 μl of 10^{-5} dilutions of H46 antimeningococcal group B globulin in borate-buffered saline (BBS), pH 8.0. The plate was incubated at 37°C for 1 h, the solution removed, and the wells washed twice with 0.05% Tween 20 in PBS. Unreacted sites were blocked with 200 μl of 1% BSA in PBS for 1 h at room temperature, and the plate was again washed twice with PBS-Tween. 100 μl of 10 μg/ml meningococcal group B CPS were added, and the plate was incubated at 37°C for 2 h, washed twice, and 100 μl of B cell culture supernatant or serum-containing antibody were added and incubated for 2 h at 37°C or overnight at 4°C. The plate was washed two or three times with PBS-Tween 20. 100 μl of a 1:2,000 dilution of alkaline phosphatase–conjugated goat anti–human IgM were added, the plate was kept at 37°C for 2 h, washed four times with PBS-Tween 20, and 0.1 ml of substrate containing 6 mg of *p*-nitrophenylphosphate in 10 ml of diethanolamine buffer, pH 9.8, were added. After 1 h at room temperature, the reaction was stopped by adding one drop of 3 N NaOH, and the plate was read at 410 nM.

ELISA for Anti-DNA Antibody. Sera, immunoglobulin fractions, and supernatants from the B cell line and its clones were tested for anti-DNA activity using an ELISA similar to that described by Shoenfeld et al. (18). The inner 60 wells of Costar EIA 96-well plates were coated with poly-L-lysine (50 μg/ml; Sigma Chemical Co., St. Louis, MO), and incubated for 2 h. After washing with PBS, native or denatured calf thymus DNA (5 μg/ml; Worthington Biochemical Corp.) was added to half of the wells, distilled water to the other half, and the plates were incubated at room temperature for 1 h. The plates were washed again and coated with poly-L-glutamate, 50 μg/ml, for 1 h. The plates were washed and could be stored at this stage for several days at 4°C. Sera or supernatants were then added in duplicate to the DNA-coated wells and control wells, and incubated 1 h. The wells were washed thoroughly with PBS-Tween, (Kirkegaard and Perry Laboratories, Inc., Gaithersberg, MD), aspirated dry, and alkaline phosphatase–conjugated goat anti–human IgM (Kirkegaard and Perry Laboratories, Inc.) was added at a 1:2,500 dilution. The plates were incubated overnight. The plates were again thoroughly washed, aspirated dry, and *p*-nitrophenylphosphate (1 mg/ml in diethanolamine buffer) was added to each well. The reaction was stopped with 5 N NaOH, and optical densities were read at 405 nm on a Bio-Tek (model EL307EIA; Bio-Tek Instruments, Inc., Burlington, VT) reader.

EBV-transformed B Cell Line and Clones. Peripheral blood lymphocytes from the patient were harvested after Ficoll-diatrazoate gradient centrifugation, rosetted with sheep erythrocytes, and the nonrosetted fraction was further depleted of T cells by treatment with anti-T3, anti-T11, and rabbit complement (19). This B cell–enriched population was then infected with EBV obtained from the supernatant of B95-8 marmoset leukocytes (American Type Culture Collection, Rockville, MD) incubated at 37°C with 5% CO_2, and fed twice weekly with Iscove's modified Dulbecco's medium (IMDM; Life Technologies, Inc.,

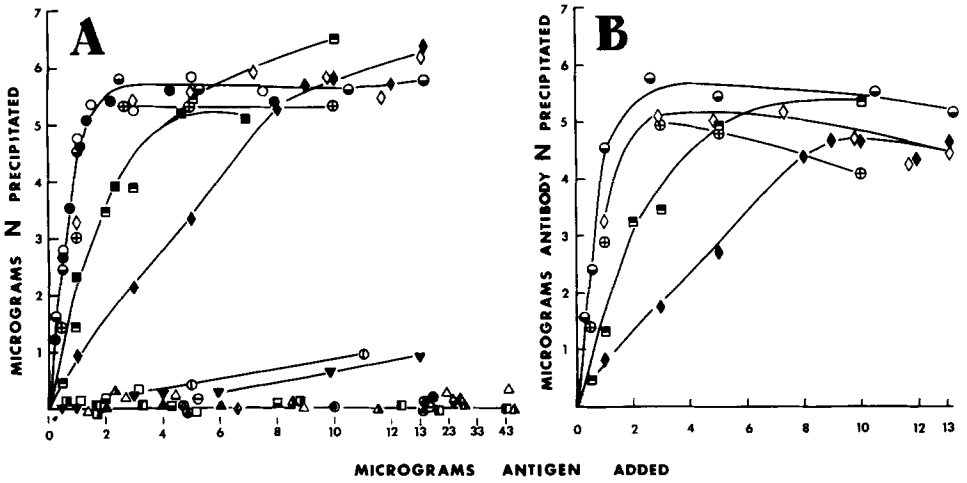

FIGURE 1. Quantitative precipitin curves for IgM^{NOV} with various polysaccharides, polynucleotides, and DNA. 15 μl of a 1:10 dilution of IgM^{NOV} in a total volume of 200 μl. (*A*) Total nitrogen precipitated. (*B*) Antibody nitrogen precipitated. ⊖, meningococcal CPS group B; ●, *E. coli* K1 CPS LH *O*-acetyl-negative; ○, *E. coli* K1 CPS 016 *O*-acetyl-negative; ◇, poly(A); ⊕, poly(I); ■, *E. coli* K235 CPS *O*-acetyl-negative; ⊟, denatured DNA; ♦, poly(G); ⊕, colominic acid; ▼, Bacto-agar, 20°C, extracted; □, *E. coli* K1 CPS LH *O*-acetyl-positive; ▲, *E. coli* K100 CPS; △, *E. coli* K92 CPS N67; ⧋, *E. coli* K92 CPS MT1389; ◧, meningococcal CPS group C; ◨, meningococcal CPS group Y; ⊟, native DNA; ⧫, chondroitin sulfate type A; ◈, chondroitin sulfate type B; ⊖, chondroitin sulfate type C; ⊛, Klebsiella K21; ⊛, Klebsiella K30; ⊙, polysialoglycoprotein.

Chagrin Falls, OH) containing 5% FCS (HyClone Laboratories, Logan, UT). After 3 wk, the cells were transferred to a flask and cloned by limiting dilution into 96-well microtiter plates (Costar, Cambridge, MA) in medium consisting of a 2:1 mixture of IMDM, and B cell–conditioned medium with 10% FCS. The B cell–conditioned medium was obtained by filtering the supernatant from an EBV-immortalized B cell line derived from a normal healthy donor. After 2–3 wk, supernatants from clones were assayed for anti-DNA activity while still growing in microtiter plates. Thereafter, they were transferred to 24-well and then to 12-well plates and assayed repeatedly for anti-DNA and antimeningococcal activity.

Phenotypic Analysis by Direct and Indirect Immunofluorescence of NOV B Cell Line. Phenotypic analysis of the B cell line was performed by indirect immunofluorescence with the mAbs OKB4 and OKIa, the monocyte markers, OKM1 and OKM2, and the T cell marker, OKT3, and fluorescein-conjugated affinity-purified $F(ab')_2$ goat anti-mouse IgG + IgM (The Jackson Laboratories, Bar Harbor, ME) using a model 30-H Cytofluorograph (Ortho Diagnostic Systems, Inc., Raritan, NJ) as described (19). Direct immunofluorescence was done using fluorescein $F(ab')_2$ fragments of goat anti-human κ and anti-human λ (free and bound light chain–specific), as well as with fluorescein-conjugated rabbit and human Ig. Fluorescein conjugates were added to 5×10^5 cells at appropriate dilutions, mixed and incubated at 4°C for 30 min. The cells were washed twice and resuspended in PBS with 0.1% sodium azide. Fluorescein-conjugated OKT3 was included as a control.

Results

Quantitative Precipitin Studies. Screening by the Ouchterlony method showed that IgM^{NOV} reacts with *E. coli* K1 and group B meningococcal CPS. We then set up quantitative precipitin curves (Fig. 1). The two preparations of group B meningococcal CPS and the two *E. coli* K1 CPS from the *O*-acetyl–negative

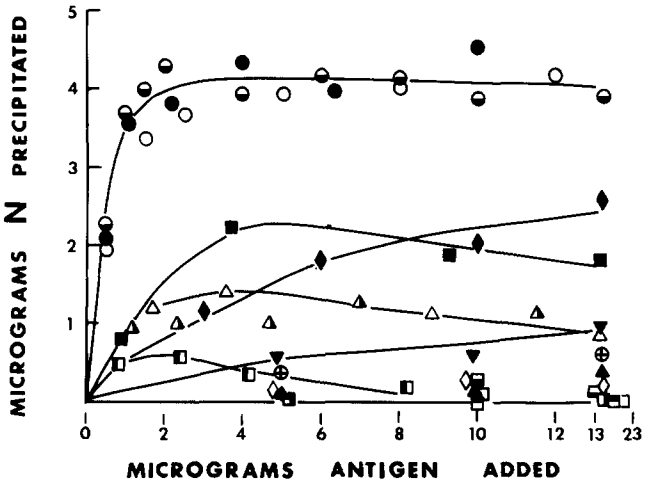

FIGURE 2. Quantitative precipitin curves for H46 with various polysaccharides, polynucleotides, and DNA. Symbols as in Fig. 1. 100 μl 1:5-diluted anti-horse 46 total volume: 200 μl.

variants of strain LH and 016 gave identical precipitin curves over a range of 2–7 μg nitrogen with these CPS. A maximum of ~5.6 μg nitrogen was precipitated from 15 μl of a 1:10 dilution of the patient's serum. This is equivalent to 23 mg of antibody per milliliter. *E. coli* CPS K235 (*O*-acetyl–negative) was ~40 percent as effective per unit weight in precipitating K1 antibodies from this serum, probably due to lower molecular mass; colominic acid reacted only slightly; the O-acetylated *E. coli* K1 strain LH gave only minimal precipitation; and there was no precipitation with K92, strains N67 and MT 1389, nor with K100.

The high degree of specificity for α(2 → 8)-linked poly-NeuNAc of which the *E. coli* K1 and meningococcal group B CPS are composed is seen by the absence of precipitation over a wide range of concentrations of other polysaccharides, including meningococcal group C, α(2 → 9)-linked poly-NeuNAc; *E. coli* K92, built of alternating α(2 → 8)α(2 → 9)-linked NeuNAc; with meningococcal group Y (NeuNAc-α(2 → 4)Glc-α(1 → 6)NeuNAc); and by the lower activity of the *E. coli* K1 *O*-acetyl–positive variant of strain LH (for elucidation of structures see Egan [20]). IgM^{NOV} also reacted with polynucleotides, poly(A) and poly(I) being as active as the two group B meningococcal and *E. coli* K1 CPS. Although native DNA did not precipitate, denatured DNA gave a good precipitin curve, as did poly(G). With denatured DNA or poly(G), more total nitrogen was precipitated than the maximum found for meningococcal group B and *E. coli* K1 polysaccharides (Fig. 1*A*), this was due to the higher nitrogen content of DNA and the polynucleotides. Fig. 1*B* shows that when the nitrogen content of the added antigens was subtracted, and antibody nitrogen precipitated was plotted against antigen added, both curves reached the same maximum; this did not occur with poly(A) and poly(I), perhaps due to slightly higher solubility of their antigen-antibody complexes. The denatured DNA was only 33% as effective per unit weight in precipitating IgM^{NOV}, and poly(A), and poly(I) were about as active (within experimental error) as the most active CPS.

Quantitative precipitin studies on H46 (Fig. 2) showed that group B menin-

TABLE I

Antibody IgM^{NOV} Protects Infant Rats against Escherichia coli K1 Infection

Exp.	LD_{50} (*E. coli* CFU/rat)	Protective activity of antisera			
		Challenge dose (LD_{50})	Antiserum	Dose (μg/rat)	Survival
1	15	0.2	IgM^{NOV}	10	10/10
		2	IgM^{NOV}	10	9/10
		20	IgM^{NOV}	10	9/10
2	5	2	IgM^{NOV}	1	8/9
		20	IgM^{NOV}	1	9/9
		200	IgM^{NOV}	1	7/9
		2	H46	0.05	6/9
		20	H46	0.05	3/9
3	6	0.3	IgM^{NOV}	1	10/10
		3.0	IgM^{NOV}	1	10/10
		33.0	IgM^{NOV}	1	9/9
		0.3	H46	1	9/10
		3.0	H46	1	10/10
		33.0	H46	1	9/9

gococcal polysaccharide and *E. coli* K1 CPS from the *O*-acetyl–negative variant of strains LH and 016 give identical precipitin curves. The *O*-acetyl–negative CPS, K235 and K92 were poorer; meningococcal group C CPS gave a minimal reaction; *E. coli* K100 and meningococcal group Y CPS gave no or barely detectable precipitation. Poly(A), poly(I), and native and denatured DNA did not react. Poly(G) reacted, but ~16 times more poly(G) was needed than with *E. coli* K1 and group B meningococcal CPS. A variety of other structurally unrelated polysaccharides did not react.

Protective Power of IgM^{NOV}. Assays of the protective power of a serum sample containing IgM^{NOV} taken September 26, 1984, and of the H46 antiserum are summarized in Table I. The LD_{50} was 5–15 CFU/animal. 1 μg of antibody (50 μl of 1:1,165 dilution), as well as 1 μg of H46 protected all infant rats against challenge with 33 LD_{50} of *E. coli* K1.

Anti-DNA Binding of Serum Samples and Immunoglobulin Fractions of IgM^{NOV}. Serum of IgM^{NOV} showed specific binding with both denatured and native DNA in ELISA (Fig. 3), but the latter was less active per unit weight. In comparable inhibition ELISA, it was ~1,000 times less active than denatured DNA at inhibiting binding to denatured DNA or to group B meningococcal CPS (data not shown). Purified IgM fractions were also similarly positive for both denatured and native DNA (Fig. 3*D*). To test whether this anti-DNA activity was due to the antimeningococcal mAb, serum was absorbed with meningococcal polysaccharide and retested. These supernatants lost reactivity with both denatured DNA and meningococcal polysaccharide (Fig. 3*C*).

To exclude nonspecific binding of IgM antibodies to denatured DNA as a possible explanation of our observations, serum from five other patients with macroglobulinemia were tested in parallel with serum NOV (Fig. 3, *A* and *B*). At low concentrations, some of these five sera bound nonspecifically to the plates,

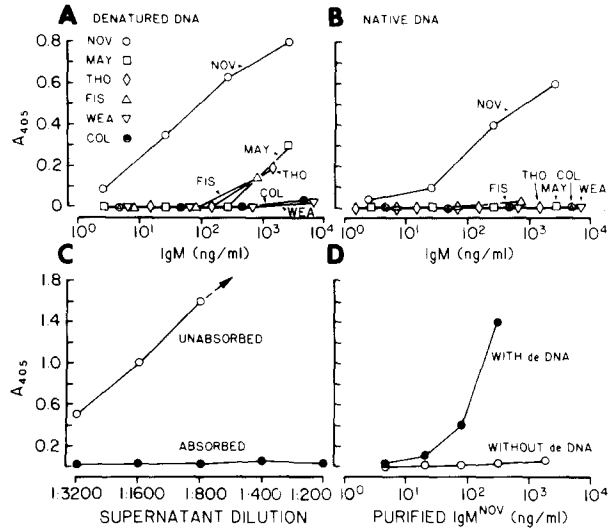

FIGURE 3. Anti-DNA ELISA of serum and purified IgM^{NOV} in nanograms of nitrogen per milliliter. NOV serum and five other sera containing monoclonal IgM proteins tested against denatured (*A*) and native (*B*) DNA by ELISA. Serum dilutions have been converted to nanograms of IgM present per milliliter, to adjust for the varying amounts of macroglobulin in each serum. Values represent mean of duplicate samples. (*C*) Anti-DNA ELISA of NOV serum before and after absorption with meningococcal polysaccharide. A 1:10 dilution of serum was added to 5.25 μg polysaccharide in a total volume of 200 μl. The dilutions shown are of the supernatants with and without polysaccharide. (*D*) Purified IgM^{NOV} with denatured (*de*) DNA as the substrate. Values shown are means of duplicates. Shaded circles indicate wells coated with DNA; open circles indicate control wells without DNA.

and two had some specific activity against denatured DNA in the range of 10^3–10^4 ng/ml. None of these five sera had any specific activity against native DNA at the dilutions tested.

Attempts to Establish B Cell Clones Making the Antibody. As a source of monoclonal IgM protein, we established several B cell lines using EBV infection, and cloned them by limiting dilution. The supernatants from all of the B cell lines were strongly positive against denatured DNA by ELISA. The anti-DNA ELISA was used to screen supernatants, as it was at least 10-fold more sensitive than the antimeningococcal assay. 4 of the 15 clones that grew at 10 cells/well had anti-DNA activity, and one of the three clones that grew at an average of 0.2 cells/well were positive. Two of these five clones that showed the strongest anti–denatured DNA activity were weakly positive by the antimeningococcus ELISA. Although these clones maintained their specificity and were expanded over several weeks, they subsequently stopped growing and with repeated feedings, the supernatants lost anti-DNA activity. The phenotype of the B cell line NOV-1 was consistent with EBV transformation rather than with that of fortuitously cultured plasma cells. It was positive for B4, Ia, and λ light chains by immunofluorescence, and was negative for monocyte markers M1 and M2, the T cell marker, T3, and κ light chains.

Discussion

The antibody specificity of myeloma proteins and macroglobulins has been of long-standing interest (21). In the course of screening sera from individuals with monoclonal gammopathies, we have identified an IgM antibody with specificity for poly-α(2 → 8)-*N*-acetylneuraminic acid, which is the CPS of meningococcus group B and *E. coli* K1. This antibody does not precipitate with poly-α(2 → 9)NeuNAc nor with *E. coli* K92 CPS with alternating α(2 → 8) and α(2 → 9)NeuNAc in quantitative precipitin studies. Absorption of serum with either group B meningococcus or *E. coli* K1 CPS completely removes the monoclonal peak. Its combining site is thus most likely directed toward an oligomer of α(2 → 8)NeuNAc. By quantitative precipitin studies, this antibody also reacts strongly with denatured DNA and the polynucleotides poly(A), poly(I), and poly(G). Poly(A) and poly(I) precipitate as well as poly-α(2 → 8)NeuNAc, and denatured DNA reacts about one-third and poly(G) one-eighth as strongly (Fig. 1). With other macroglobulins tested here and previously (22), concentrations ~100 times higher are required to show similar levels of binding with denatured DNA. Minimal reactivity with native DNA was detectable both by direct and inhibition ELISA, but not by quantitative precipitin tests.

The crossreactivity of this antibody with denatured and native DNA, and with the polynucleotides poly(A), poly(I), and poly(G), was unanticipated. Theoretically, one might be concerned that the antibody might cause symptoms of SLE if used in vivo, just as others (4, 5) have been concerned that antimeningococcal vaccines might give rise to troublesome antibodies crossreacting with the gangliosides GM_3 and GD_3, which contain oligomers of poly-α(2 → 8)NeuNAc. This particular antibody, however, does not crossreact with a mixture of gangliosides or with purified GM_3 (L. Freddo, and N. Latov, personal communication), and in vivo at a concentration of 23 mg/ml, it has not caused signs or symptoms of SLE.

Frosch et al. (23) have recently shown that, on injection of live meningococci, autoimmune NZB mice can mount an IgG response to α(2 → 8)NeuNAc, in contrast to normal BALB/c mice, which make only low levels of IgM. They have exploited this finding to isolate an IgG2a mAb specific for α(2 → 8)NeuNAc from an NZB mouse. It is interesting in the light of our findings of IgM^{NOV} reactivity with both α(2 → 8)NeuNAc and denatured DNA, that NZB mice, as they mature, show increasingly high titers of IgM and IgG anti-DNA.

The activity of this antibody for denatured DNA also has relevance to recent studies of anti-DNA mAbs isolated from patients with SLE. Numerous examples of crossreactivity have been described among both human and murine anti-DNA antibodies, including those with various polynucleotides (24–26), phospholipids (26), cell-surface polypeptides (27), and proteoglycans (28). These crossreactivities have been invoked in some cases to account for the protean serological and clinical manifestations of SLE. Close scrutiny of anti-DNA antibodies has also revealed some instances in which they appear to be closely related to antibacterial antibodies (29). In one instance, a mutation giving rise to a single amino acid difference in a mouse IgA myeloma heavy chain transformed an antiphosphorylcholine antibody into an antibody with specificity for double-stranded DNA (30). In addition, the 16/6 idiotype, specific for an anti-DNA mAb, not only identifies

many lupus sera (25), but also reacts with IgM macroglobulins (22), which are specific for Klebsiella CPS with pyruvylated galactose (6). Part of the amino acid sequence of the heavy and light chain variable regions of four 16/6-positive anti-DNA antibodies has been determined (31), and the κ light chains are homologous to that of IgM^{WEA}, which reacts with Klebsiella polysaccharide K30.

The molecular basis of these crossreactivities between lupus antibodies to DNA and phospholipids, glycosaminoglycans, and cell surface proteins, as well as those between the anti-Klebsiella macroglobulins and polynucleotides and their reactivity with the 16/6 idiotype remains unknown. Others (26) have suggested that phosphodiester groups with a given spacing might constitute a crossreacting antigenic determinant between DNA and phospholipids. For bacterial polysaccharides, a similar pattern of negative charges is recognized by closely related antibodies. With IgM^{NOV}, which recognizes DNA as well as poly-α(2 $\rightarrow$ 8)NeuNAc, but not poly-α(2 $\rightarrow$ 9)NeuNAc or alternating poly-α(2 $\rightarrow$ 8)α(2 $\rightarrow$ 9)NeuNAc, the only structural feature to account for both reactivities would be a similarity in the spatial distribution of charges between the carboxyl groups of the poly-α(2 $\rightarrow$ 8)NeuNAc and the phosphates of the denatured DNA or poly(A) and poly(I). The charge distribution of the poly-α(2 $\rightarrow$ 9)NeuNAc and the poly-α(2 $\rightarrow$ 8)α(2 $\rightarrow$ 9)NeuNAc would be quite different, and so would support this hypothesis. Indeed Lindon et al. (32) have shown substantial conformational differences between poly-α(2 $\rightarrow$ 8)NeuNAc (group B) and poly-α(2 $\rightarrow$ 9)NeuNAc (group C) by ^3H nuclear magnetic resonance spectroscopy, indicating different three-dimensional structures in solution. Heidelberger (33) has interpreted the extensive crossreaction between types 8 and 19 pneumococcal polysaccharides as due to the negatively charged phosphoryl-β-D-ManNAc of type 19 being able to enter the type 8 site, which is specific for cellobiuronic acid with its carboxyl, and vice versa. Thus, an important facet of crossreactivity to charged groups is the apparent association of similarities and differences in charge distribution being responsible for immunological crossreactions among what have been generally considered diverse and structurally unrelated substances.

IgM^{NOV} is protective in rats, and thus of potential use in serotherapy of meningococcal group B and certain invasive *E. coli* infections. For reasons that remain poorly understood, the poly-α(2 $\rightarrow$ 8)NeuNAc that makes up the capsular polysaccharide of these organisms is weakly immunogenic in normal mice as well as in humans (1, 3, 34). Group B meningococcal strains account for 70–80% of endemic disease caused by meningococci in developed countries, of which at least 10% are fatal, despite antibiotics (35). Deafness and other neurological sequelae frequently result. K1 strains account for ~80% of neonatal *E. coli* sepsis and meningitis, and ~11–25% of isolates from the blood of adult patients (36). In the preantibiotic era, as early as 1900, horse antimeningococcal serum was used in the treatment of cerebrospinal meningitis, by injection into the subarachnoid space, with surprisingly good results, especially considering the problems of typing meningococci (37, 38). The antibody was almost certainly IgM, based on subsequent studies of horse antisera to pneumococcal and other polysaccharides (see 39). Moreno et al. (9, 40) have also shown that monoclonal IgMs specific for meningococcal group B CPS are protective in mice, and Kim et al. (41) have shown that murine mAb is effective in established *E. coli* K1 infections. Group B

antibody may be produced by injection of formalized meningococci in animals, but whole meningococci are too toxic to permit their use in humans. There is active investigation in developing a group B meningococcal vaccine (9, 40, 42), which might be useful in populations such as military recruits. Even if such efforts are successful, use of such a vaccine in groups not at high risk is not necessarily warranted. In clustered outbreaks of disease in the general population, however, group B–specific human antiserum as well as mAb might be supplementary treatment for those infected.

In the attempt to produce human mAbs in vitro, we have immortalized B cells from this patient and have shown that relevant antibody-producing cells are present in these lines. Moreover, by limiting dilution, we have derived clones of B cells secreting this antibody. To date, however, these clones have been unstable with respect to growth and antibody secretion. Nevertheless, that we have been able to grow B cell clones secreting the myeloma protein from peripheral blood with EBV is direct evidence of a circulating population of B cell precursors to malignant plasma cells, and provides additional evidence to that derived by studies with antiidiotypic antibodies that an expanded pool of such cells exists in the peripheral blood of patients with plasma cell dyscrasias (43).

Summary

We have described an IgM antibody from a patient with macroglobulinemia specifically reacting with poly-α(2 → 8)*N*-acetyl neuraminic acid (NeuNAc) the capsular polysaccharide of two important human pathogens, group B meningococcus and *E. coli* K1. This antibody has a narrowly defined specificity in its interactions with polysaccharides, being unable to bind poly-α(2 → 9)NeuNAc or alternating poly-α(2 → 8)α(2 → 9)NeuNAc. However, it shows interesting crossreactivity with seemingly unrelated polynucleotides and denatured DNA, supporting the hypothesis that charged groups with a given spacing may determine the specificity of antigen-antibody interactions on otherwise dissimilar molecular structures. Despite the crossreactivity with denatured DNA and polynucleotides, the antibody does not appear to have adverse effects in the patient. The antibody protects newborn rats against *E. coli* K1 infection, as well as the standard horse antiserum H46, and one would expect it to prove useful in humans as an adjunct to antibiotic therapy in infections with group B meningococcus and *E. coli* K1. We have attempted to clone the antibody-producing cells from peripheral blood, and have shown that the relevant cells are present and can be cultured.

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References

1. Kasper, D. L., J. Winkelhake, W. D. Zollinger, B. L. Brandt, and M. S. Artenstein. 1973. Immunochemical similarity between polysaccharide antigens of *Escherichia coli* 07:K1(L):NM and group B *Neisseria meningitidis*. *J. Immunol.* 110:262.
2. Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Orskov, I. Orskov, and L.

A. Hanson. 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. *N. Engl. J. Med.* 290:1216.

3. Wyle, F. A., M. S. Artenstein, B. L. Brandt, E. C. Traumont, D. L. Kasper, P. L. Alteri, S. L. Berman, and J. P. Lowenthal. 1972. Immunologic response of man to Group B meningococcal polysaccharide vaccine. *J. Infect. Dis.* 126:514.
4. Finne, J., M. Leinonen, and P. H. Makela. 1983. Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development. *Lancet.* ii:235.
5. Soderstrom, T., G. Hansson, and G. Larson. 1984. The *Escherichia coli* K1 capsule shares antigenic determinants with the human gangliosides GM3 and GD3. *N. Engl. J. Med.* 310:726.
6. 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.
7. Kabat, E. A., J. Liao, W. H. Sherman, and E. F. Osserman. 1984. Immunochemical characterization of the specificities of two human monoclonal IgM's reacting with chondroitin sulfate. *Carbohydr. Res.* 30:289.
8. Barry, G. T. 1958. Colominic acid a polymer of *N*-acetylneuraminic acid. *J. Exp. Med.* 107:507.
9. Moreno, C., M. R. Lifely, and J. Esdaile. 1985. Immunity and protection of mice against *Neisseria meningitidis* group B by vaccination, using polysaccharide complexed with outer membrane proteins: A comparison with purified B polysaccharide. *Infect. Immun.* 47:527.
10. Orskov, F., I. Orskov, A. Sutton, R. Schneerson, W. Lin, W. Egan, G. E. Hoff, and J. B. Robbins. 1979. Form variation in *Escherichia coli* K1: Determined by *O*-acetylation of the capsular polysaccharide. *J. Exp. Med.* 149:669.
11. Allen, P. Z., M. Glode, R. Schneerson, and J. B. Robbins. 1982. Identification of immunoglobulin heavy-chain isotypes of specific antibodies of horse 46 group B meningococcal antiserum. *J. Clin. Microbiol.* 15:324.
12. Glode, M. P., A. Sutton, E. R. Moxon, and J. B. Robbins. 1977. Pathogenesis of neonatal *Escherichia coli* meningitis: Induction of bacteremia and meningitis in infant rats fed *E. coli* K1. *Infec. Immun.* 16:75.
13. Brodeur, B. R., Y. Larose, P. Tsang, J. Hamel, F. Ashton, and A. Ryan. 1985. Protection against infection with *Neisseria meningitidis* group B serotype 2b by passive immunization with serotype-specific monoclonal antibody. *Infect. Immun.* 50:510.
14. Bortolussi, R., and P. Ferrieri. 1980. Protection against *Escherichia coli* K1 infection in new born rats by antibody to K1 capsular polysaccharide antigen. *Infect. Immun.* 28:111.
15. Ouchterlony, O. 1953. Antigen-antibody reaction in gels IV. Types of reactions in coordinated systems of diffusion. *Acta Pathol. Microbiol. Scand.* 32:231.
16. Kabat, E. A. 1980. Basic principles of antigen-antibody reactions. *Methods Enzymol.* 70:3.
17. Schiffman, G., E. A. Kabat, W. Thompson. 1962. Immunochemical studies on blood groups. XXVII. Periodate oxidation of blood group A, B, and O(H) substances. *J. Am. Chem. Soc.* 84:463.
18. Shoenfeld, Y., S. C. Hsu-Lin, J. E. Gabriels, L. E. Silberstein, B. C. Furie, B. Furie, B. D. Stollar, and R. S. Schwartz. 1982. Production of autoantibodies by human-human hybridomas. *J. Clin. Invest.* 70:205.
19. Thomas, Y., L. Rogozinski, O. H. Irigoyen, H. H. Shen, M. A. Talle, G. Goldstein,

and L. Chess. 1982. Functional analysis of human T cell subsets defined by monoclonal antibodies. *J. Immunol.* 128:1386.

20. Egan, W. 1980. Structure of the polysaccharide antigens from *Haemophilus influenzae* and *Neisseria meningitidis* by ^{13}C NMR spectroscopy. *In* Magnetic Resonance in Biology. J. S. Cohen, editor. 1:197.
21. Seligmann, M., and J C. Brouet. 1973. Antibody activity of human myeloma globulins. *Semin. Hematol.* 10:163.
22. Naparstek, Y., D. Duggan, A. Schattner, M. P. Madaio, F. Goni, B. Frangione, B. D. Stollar, E. A. Kabat, and R. S. Schwartz. 1985. Immunochemical similarities between monoclonal antibacterial Waldenstrom's macroglobulins and monoclonal anti-DNA lupus autoantibodies. *J. Exp. Med.* 161:1525.
23. Frosch, M., I. Gorgen, G. J. Boulnois, K. N. Timmis, and D. Bitter-Suermann. 1985. NZB mouse system for production of monoclonal antibodies to weak bacterial antigens: Isolation of an IgG antibody to the polysaccharide capsules of Escherichia coli K1 and group B meningococci. *Proc. Natl. Acad. Sci. USA.* 82:1194.
24. Shoenfeld, Y., J. Rauch, H. Massicotte, S. K. Datta, J. Andre'-Schwartz, B. D. Stollar, and R. S. Schwartz. 1983. Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. *N. Engl. J. Med.* 308:414.
25. Shoenfeld, Y., D. A. Isenberg, J. Rauch, M. P. Madaio, B. D. Stollar, and R. S. Schwartz. 1983. Idiotypic cross-reactions of monoclonal human lupus autoantibodies. *J. Exp. Med.* 158:718.
26. Lafer, E. M., J. Rauch, C. Andrzejewski, D. Mudd, B. Furie, B. Furie, R. S. Schwartz, and B. D. Stollar. 1981. Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J. Exp. Med.* 153:897.
27. Jacob, L., M.-A. Lety, D. Louvard, and J. F. Bach. 1985. Binding of a monoclonal anti-DNA antibody to identical protein(s) present at the surface of several human cell types involved in lupus pathogenesis. *J. Clin. Invest.* 75:315.
28. Faaber, P., P. J. A. Capel, G. P. M. Rijke, G. Vierwinden, L. B. A. Van de Putte, and R. A. P. Koene. 1984. Cross-reactivity of anti-DNA antibodies with proteoglycans. *Clin. Exp. Immunol.* 55:502.
29. Eilat, D., M. Hochberg, J. Pumphrey, and S. Rudikoff. 1984. Monoclonal antibodies to DNA and RNA from NZB/NZW F_1 mice: Antigenic specificities and NH_2-terminal amino acid sequences. *J. Immunol.* 133:489.
30. Diamond, B., and M. D. Scharff. 1984. Somatic mutation of the T15 heavy chain gives rise to an antibody with autoantibody specificity. *Proc. Natl. Acad. Sci. USA.* 81:5841.
31. Atkinson, P. M., G. W. Lampman, B. C. Furie, Y. Naparstek, R. S. Schwartz, B. D. Stollar, and B. Furie. 1985. Homology of the NH_2-terminal amino acid sequences of the heavy and light chains of human monoclonal lupus autoantibodies containing the dominant 16/6 idiotype. *J. Clin. Invest.* 75:1138.
32. Lindon, J. C., J. G. Vinter, M. R. Lifely, and C. Moreno. 1984. Conformational and dynamic differences between *N. meningitidis* serogroup B and C polysaccharides, using N.M.R. spectroscopy and molecular mechanics calculations. *Carbohydr. Res.* 13:59.
33. Heidelberger, M. 1983. Precipitating cross-reactions among pneumococcal types. *Infect. Immun.* 41:1234.
34. Weinstein, R., and L. S. Young. 1978. Phagocytic resistance of *Escherichia coli* K1 isolates and relationship to virulence. *J. Clin. Microbiol.* 8:748.
35. Geiseler, P. J., K. E. Nelson, S. Levin, K. T. Reddi, and V. K. Moses. 1980. Community-acquired purulent meningitis: A review of 1,316 cases during the antibiotic era, 1954–1976. *Rev. Infect. Dis.* 2:725.

36. Pitt, J. 1978. K1 antigen of *Escherichia coli*: Epidemiology and serum sensitivity of pathogenic strains. *Infect. Immun.* 22:219.
37. Blackfan, K. D. 1922. The treatment of meningococcus meningitis. *Medicine.* 1:140.
38. Gold, R. 1985. Prevention of bacterial meningitis by immunological means. *In* Bacterial Meningitis. M. A. Sande, A. L. Smith, and R. K. Root, editors. Churchill-Livingstone, London.
39. Kabat, E. A. 1961. Kabat and Mayer's Experimental Immunochemistry. Second edition. Chas. C. Thomas, Inc., Springfield, IL.
40. Moreno, C., J. Hewitt, K. Hastings, and D. Brown. 1983. Immunological properties of monoclonal antibodies specific for meningococcal polysaccharides: the protective capacity of IgM antibodies specific for polysaccharide group B. *J. Gen. Microbiol.* 129:2451.
41. 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.
42. Zollinger, W. D., R. E. Mandrell, J. M. Griffiss, P. Altieri, and S. Berman. 1979. Complex of meningococcal group B polysaccharide and type 2 outer membrane protein immunogenic in man. *J. Clin. Invest.* 63:836.
43. Kubagawa, H, L. B. Vogler, J. D. Capra, M. E. Conrad, A. R. Lawton, and M. D. Cooper. 1979. Studies on the clonal origin of multiple myeloma. *J. Exp. Med.* 150:792.