THE EPITOPE ASSOCIATED WITH THE BINDING OF THE CAPSULAR POLYSACCHARIDE OF THE GROUP B MENINGOCOCCUS AND OF *ESCHERICHIA COLI* K1 TO A HUMAN MONOCLONAL MACROGLOBULIN, IgM$^{\text{NOV}}$

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A human monoclonal macroglobulin IgM$^{\text{NOV}}$, has recently been described which has specificity for poly-$\alpha(2\rightarrow8)$-linked N-acetylneuraminic acid (NeuNAc),[1] the specific capsular polysaccharide of the group B meningococcus and of *Escherichia coli* K1 (1). Although studies using this polysaccharide as a vaccine in humans have been unsuccessful (2–4, see reference 5), the serum contained over 20 mg of antibody per milliliter. Quantitative precipitin curves of IgM$^{\text{NOV}}$ with four preparations, two of group B meningococcal polysaccharide and two of *E. coli* K1 were identical (1). IgM$^{\text{NOV}}$ did not precipitate with polysaccharides from group C meningococci composed of poly-$\alpha(2\rightarrow9)$NeuNAc nor with *E. coli* K92 built of alternating $\alpha(2\rightarrow8)\alpha(2\rightarrow9)$NeuNAc, nor with many other polysaccharides.[2] Surprisingly, the polynucleotides poly(A) and poly(I) gave precipitin curves per unit weight identical with those of poly-$\alpha(2\rightarrow8)$NeuNAc. Denatured DNA and poly(G) also crossreacted, but about 3 and 12 times as much, respectively, was needed to precipitate comparable amounts of antibody nitrogen (N) from IgM$^{\text{NOV}}$(1). These crossreactivities would appear to correspond to the findings of Heidelberger (6) who 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 antibody combining site which is specific for cellobiuronic acid with its carboxyl and vice versa. It was postulated (1) therefore, that the crossreactions with poly(A), poly(I) and denatured DNA could be accounted for by "a similarity in the spatial distribution of charges between the carboxyl groups of poly-$\alpha(2\rightarrow8)$NeuNAc and the phosphates of the denatured DNA or poly(A) and poly(I)" (1).

The combining sites of mAbs to α(1→6)dextran have been classified into cavity-type sites (7), also termed end binders (8), in which the terminal nonreducing one or two sugars contribute most of the binding energy with additional binding energy indicating a site size for W3129 complementary to five α(1→6)linked glucoses, and groove-type sites in which the combining site is complementary to an internal linear sequence of up to six or seven sugars not involving the nonreducing end (7, 9); other antibodies behave similarly with site sizes complementary to 4 to 7 sugars (9-11).

Poly-α(2→8)NeuNAc carbohydrate antigens and their antibodies behaved differently. Lindon et al. (12) noted by ^{3}H–nuclear magnetic resonance (NMR) spectroscopy that group B polysaccharide tumbles as a rigid species with internal rotation only at C9 of its side chains whereas the group C polysaccharide shows internal or segmental motion in the C7 to C9 side chains of the sialic acids. Jennings et al (13) found that, with horse anti–group B meningococcal serum, H46, higher molecular weight oligosaccharides, e.g., a decasaccharide were required to inhibit binding to intact group B polysaccharide, and Finne and Mäkela (14) also found a decasaccharide was needed for direct binding. This was ascribed to the internal sugars of the group B epitope being in an unusual conformation. Subsequent NMR studies by Michon et al. (15) on α(2→8)-linked NeuNAc oligosaccharides of increasing sizes established that an α(2→8)-linked pentamer is required before a linkage similar to the internal linkages of colominic acid is generated.

The horse antiserum did not react with poly(A), poly(I), native, or denatured DNA, but crossreacted weakly with poly(G) (1). Monoclonal IgM^NOV and polyclonal H46 were approximately of equal potency in protecting infant rats against challenge with virulent *E. coli* (1). A mouse IgG2a mAb, mAb735, to the group B polysaccharide also did not crossreact with polynucleotides and denatured DNA (16). Another monoclonal anti–meningococcal group B recognized the embryonic neural cell adhesion molecule (NCAM) but not the adult form (17). Several other mouse mAbs to the group B polysaccharide have been reported, but reactivity with polynucleotides and DNA has not yet been studied (18).

The present study attempts to define the size, shape, and conformation of the epitope of poly-α(2→8)NeuNAc most complementary to IgM^NOV to account for the crossreactivity with poly(A), poly(I), and denatured DNA and the differences in the epitopes reacting with IgM^NOV and H46.

Materials and Methods

Materials. The high molecular weight group B meningococcal polysaccharide preparations (GBMP) (1) were gifts of Drs. John Robbins of the National Institutes of Health, Bethesda, MD; Carlos Moreno then at Wellcome Research Laboratories, Beckenham, UK (3); and Dr. E. C. Gotschlich of The Rockefeller University, New York, NY (lot XIX; reference 19). Colominic acid (*E. coli*) and BSA were obtained from Sigma Chemical Co., St. Louis, MO. Preparation of the *N*-propionylated (*N*-Pr)-GBMP (20) and the covalent linking of GBMP to BSA were carried out as previously described (21). The α(2→8)-homosioalooligosaccharides, α(2→8)NeuNAc)_n were obtained by separation on an ion-exchange column of a partial hydrolysate of colominic acid (13) and the equivalent *N*-propionylated oligosaccharides (α(2→8)NeuNPr)_n were obtained using the same procedures. All α(2→8)-poly- and oligosaccharides were used as the sodium salts. Poly(A) was obtained from Boehringer-Mannheim Biochemicals, Indianapolis, IN.

A small molecular weight α(2→8)-linked sialic acid polymer was obtained by fractionation of colominic acid on a Sephadex G100 column (1.6 × 90 cm.) (Pharmacia Fine Chemicals,

Piscataway, NJ) using PBS as eluant. A narrow fraction corresponding to K^d 0.35 in the elution profile was collected and desalted on a Sephadex G10 column (Pharmacia Fine Chemicals) using water as eluant, and was lyophilized. This fraction was shown to contain ~20 $\alpha(2\rightarrow8)$-linked sialic acid residues, $(NeuNAc)_{\sim20}$ (22), which corresponds to an average molecular weight of 6,000–7,000. Delipidated GBMP was obtained using two methods: (a) The high molecular weight GBMP (10 mg) was treated with phospholipase A2 (EC 3.1.1.4) (2.2 U) (Sigma Chemical Co.) according to published procedures of Gotschlich et al. (19). Part of the solution was extracted with an equal volume of 80% phenol at room temperature to remove the enzyme and the aqueous phase was lyophilized. That the product was completely deaggregated was ascertained by the absence of a peak normally associated with the high molecular weight GBMP in the void volume of its elution profile when eluted from a Sepharose 4B column (19, 22). A small aliquot of the original solution above was acidified and extracted with chloroform, and the presence of lipids in the chloroform solution was determined by GC-MS (19). A similar deaggregation of the high molecular weight GBMP was not observed when it was treated with phospholipase C (EC 3.1.4.3) (61 U) (Sigma Chemical Co.). (b) High molecular weight GBMP (7.5 mg) was treated with 0.1 M NaOH (1 ml) for 16 h at 37°C. The solution was neutralized with 0.2M HCl, dialyzed, and lyophilized to yield the delipidated GBMP (7.2 mg). The product had a similar elution profile to that of the phospholipase A2–treated GBMP when eluted from a Sepharose 4B column.

Radioactive Antigen-binding Assay. This assay was carried out by a modification of the Farr technique (23) using an extrinsically ^{3}H-labeled GBMP (13). The reaction mixture for the radioactive antigen-binding assay consisted of mixing in Eppendorf polypropylene microtest tubes (Brinkmann Instruments Inc., Westbury, NJ) 50 µl of a solution containing 12.5 ng of ^{3}H-labeled GBMP (100 cpm/ng), increasing amounts (0–100 µl) of antisera, and sufficient PBS to take the total volume in the tubes to 150 µl. After incubation at 4°C for 16 h, 150 µl of saturated (at 4°C) ammonium sulfate or 150 µl of 5% polyethylene glycol (7,000) (Sigma Chemical Co.) in PBS at 4°C was added to the tubes and they were agitated and left to stand at 4°C for 30 min. The tubes were centrifuged at 15,000 rpm for 10 min and two aliquots of 130 µl were withdrawn from each tube. The aliquots were mixed with 5 ml of an aqueous scintillant containing xylene (Amersham Corp., Oakville, Ontario, Canada) and the mixtures were counted in an LKB 1217 Rackbeta liquid scintillation counter.

Radioactive Binding Inhibition Assay. Increasing concentrations of inhibitor in PBS (80 µl) were added to 20 µl of a 1:500 dilution of human IgM^{NOV} sufficient to bind 50% of the ^{3}H-labeled GBMP in the absence of inhibitor. The samples were then processed as described above in the radioactive antigen-binding assay using the 5% polyethylene glycol solution to precipitate the antibody. The percent inhibition was calculated using the following formula: percent inhibition = 100 × [(cpm with inhibitor − cpm without inhibitor)/(cpm without antibody − cpm without inhibitor)].

ELISA for Binding of Antibody. The wells of Linbro EIA microtiter plates (Flow Laboratories, Mississauga, Ontario, Canada) were coated by using a 10-µg/ml solution of the high molecular weight GBMP in 0.05 M sodium carbonate–bicarbonate buffer at pH 9.6 (100 µl/well) at 37°C for 3 h or by using a 10 µg/ml solution of GBMP-BSA conjugate in PBS (100 µl/well) for 3 h at room temperature. All plates were then kept for 16 h at 4°C. The plates were blocked with 1% BSA in PBS for 10 min at room temperature. The wells were then filled with 100 µl of serial 10-fold dilutions of antisera in BSA with 0.02% Tween 20, and the plates were left for 1 h at room temperature. After washing, 50 µl of a 1:200 dilution in PBS of peroxidase-conjugated goat anti–human IgM or peroxidase-conjugated goat anti–horse IgM (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) were added to the wells and the plates were incubated for 1 h at room temperature.

2,2′-Azino-di-[3-ethylbenzthiazoline sulfonate] in cacodylate buffer with hydrogen peroxide was used as the substrate and after 10 min the optical density at 414 nm was read using a Titertek Multiscan M.C. (Flow Laboratories, Meckenheim, Federal Republic of Germany).

ELISA for Inhibition of Antibody Binding. A Corning 96-well round-bottomed plate was treated with 100 µl of 0.1 M bicarbonate buffer pH 9.6 at room temperature (RT) for 1 h. The solution was removed and wells were washed three times with 0.05% Tween 20 in PBS. The wells were then coated with 100 µl of poly-L-lysine (50 µg/ml, Sigma Chemical Co.) for 2 h at room

temperature. After washing with PBS-Tween, 100 μl of GBMP (3) (1 μg/well) or poly(A) (1 μg/well) were added to the wells, and the plates were left at RT for 1 h. The plates were washed, blocked with 100 μl of poly-L-glutamate (50 μg/ml) at RT for 1 h, and again washed. 50 μl of inhibitor and 50 μl of a 2 × 10^{-4} dilution in PBS of IgM^{NOV} antiserum were added. The contents of the wells were mixed by gentle tapping and the plate was placed at RT for 1 h or at 4°C overnight. The wells were then washed thoroughly with PBS-Tween 20, and 100 μl of a 1:2,000 dilution in PBS of alkaline phosphatase-labeled goat anti-human IgM (Cappel Laboratories, Malvern, PA) was added. The plate was again thoroughly washed, and 100 μl of 0.06% p-nitrophenylphosphate in diethanolamine buffer, pH 9.8, was added as substrate. The reaction was stopped with 25 μl 3N NaOH, and optical densities were read at 410 nm.

Results

IgM^{NOV} and H46 were found to differ significantly in the Farr (23) RIA. H46 was precipitated normally by ammonium sulfate but IgM^{NOV} was not (data not shown). Fig. 1 shows that substitution of polyethylene glycol in the RIA precipitated IgM^{NOV} but did not precipitate H46. Thus, polyethyleneglycol was used in RIA with Ig-M^{NOV} and ammonium sulfate for H46. The reason for this difference is not known.

Fig. 2 shows ELISA titrations of serial dilutions of IgM^{NOV} and H46 against the high molecular weight GBMP and against a mouse anti-CFA serum as a control. The binding curves of IgM^{NOV} and H46 are identical.

Fig. 3 shows the results by RIA of assays using the (α(2→8)NeuNAc)$_n$ oligosaccharides to inhibit the binding of [^{3}H]GBMP to IgM^{NOV}. Fig. 4, A and B show the results of ELISA assays using the (α(2→8)NeuNAc)$_n$ oligosaccharides to inhibit the binding of IgM^{NOV} to GBMP and to poly(A), respectively. The inhibition curves of all three figures with the various oligosaccharides resemble each other in that oligosaccharides larger than six or seven show strikingly increased inhibition. Similar findings were obtained previously with H46 (13). This is unlike findings in other carbohydrate-anticarbohydrate systems (24) in which depending upon the system, the polyclonal or the monoclonal antibody, maximum inhibition has generally been found with four to seven sugars, larger oligosaccharides having equivalent inhibiting power on a molar basis. It has been possible to estimate sizes of antibody combining sites as that of the smallest oligosaccharide giving maximum inhibition.

For example, an IgM anti-α(1→6)dextran hybridoma,58.2C10.3 was inhibited better by IM4 than by IM3 (isomaltotriose), which was better than IM2 (isomal-
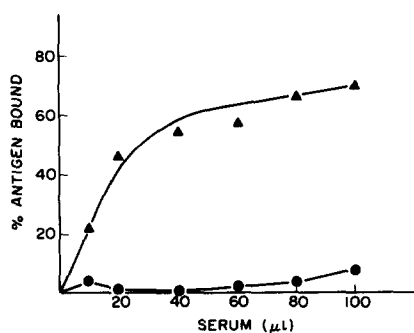

Figure 1.   Radioactive antigen-binding assay standard curves ^{3}H-labeled GBMP with horse 46 IgM (●) and human monoclonal IgM^{NOV} (▲) using polyethylene glycol as the precipitating agent.
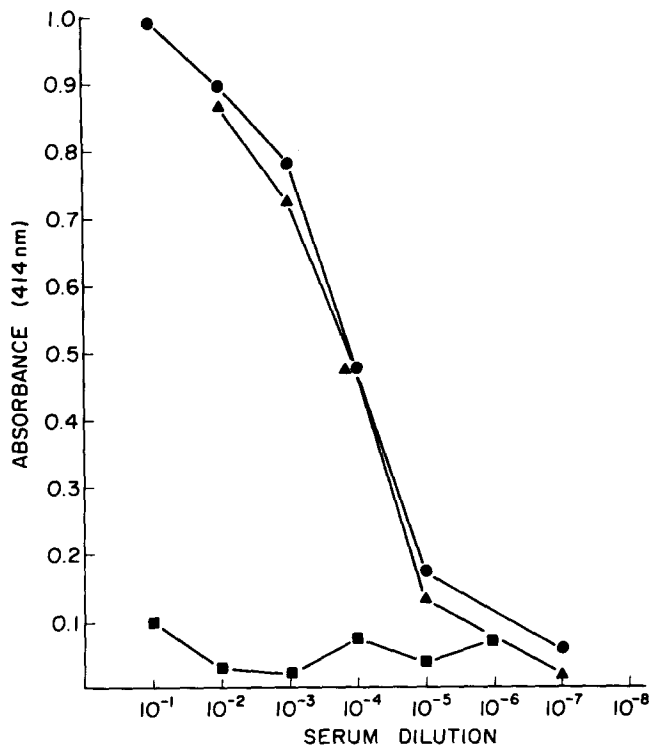
FIGURE 2. ELISA titrations of IgM^{NOV} (▲) and horse IgM (●) against the high molecular weight GBMP. A mouse anti-CFA serum (□) was used as the control serum. Curves represent the mean titrations.

tose), but equimolar concentrations of IM9, IM8, IM7, IM6, and IM5 all were identical, falling on the same line in inhibiting potency to the site filling IM4 (10). Similarly, with the IgA anti-$\alpha(1\rightarrow6)$dextran hybridoma 37.1E.5, IM9, IM8, and IM7 all required 100 nmol for 50% inhibition of precipitation of the antibody by dextran, whereas 190, 380, and 1,050 nmol of IM6, IM5, and IM4 were needed for 50%
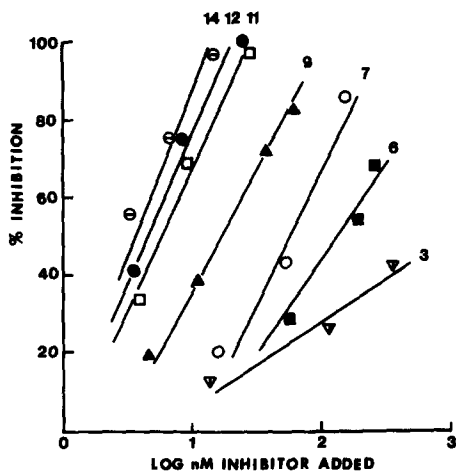

FIGURE 3. Inhibition of the binding of ^{3}H-labeled GBMP to human IgM^{NOV} using a series of linear $\alpha(2\rightarrow8)$-linked homosialooligosaccharides ($\alpha2\rightarrow8$ NeuNAc)$_n$.
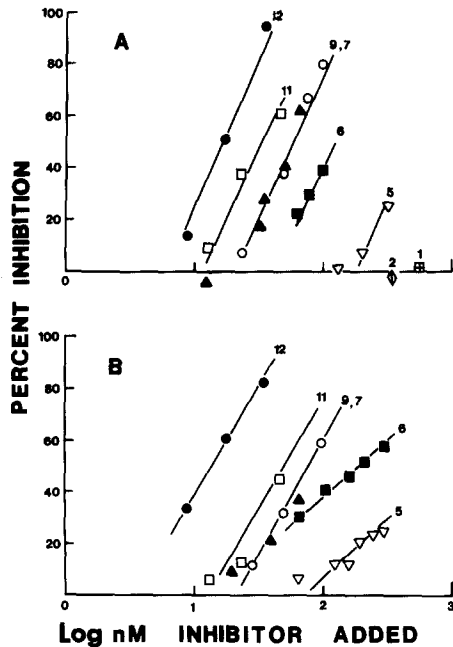
FIGURE 4. ELISA inhibitions by α(2→8)-linked NeuNAc oligosaccharides of the binding of IgM^{NOV} antibody to GBMP (A) or to poly(A) (B).

inhibition. These findings are clearly unlike those obtained by RIA (Fig. 3) and ELISA (Fig. 4, A and B) for inhibition with both the GBMP and poly(A). Comparison of Fig. 4, A and B shows that the capacities of the (α(2→8)NeuNAc)$_n$ oligosaccharides by ELISA to inhibit binding of IgM^{NOV} are remarkably similar, inhibition being first seen over the range studied with α(2→8)-linked (NeuNAc)$_5$; increasing inhibition was seen as oligosaccharide size increased up to α(2→8)-linked (NeuNAc)$_{12}$. It is especially interesting that the inhibiting potencies of α(2→8)-linked (NeuNAc)$_7$ and (NeuNAc)$_9$ fall on the same line whether the antibody of IgM^{NOV} was being displaced from GBMP or poly(A). This would suggest seven α(2→8)-linked NeuNAc units as filling the site. The increased potency of the (α(2→8)NeuNAc)$_{11}$ and (α(2→8)NeuNAc)$_{12}$ could be ascribable to these oligomers being able to fit into a groove-type antibody combining site in several ways or, in view of the NMR data, that the larger oligomers and GBMP exist in an unique conformation (15). Especially significant in this connection may be the findings in Fig. 4, A and B that the slopes of the inhibition lines of the oligomers competing against GBMP are identical, whereas in the competition with poly(A), poly-α(2→8)-linked (NeuNAc)$_5$ and (NeuNAc)$_6$ have much lower slopes than the larger oligomers that have about the same slopes. This increase in slope with poly-α(2→8)-linked (NeuNAc)$_7$, indicating increased effectiveness as an inhibitor, would be consistent with it being the smallest oligomer that could exist in a site-filling conformation or that had a sufficient number of internal residues in the preferred conformation required for reaction with the group B antibody. In Fig. 3 the slope difference between α(2→8)-linked(NeuNAc)$_3$ and (NeuNAc)$_7$ is also striking and an increase in slope is also seen between (NeuNAc)$_6$ and (NeuNAc)$_7$; why (NeuNAc)$_9$ is more active in RIA than (NeuNAc)$_7$ whereas in ELISA it is identical is not clear, but conceivably

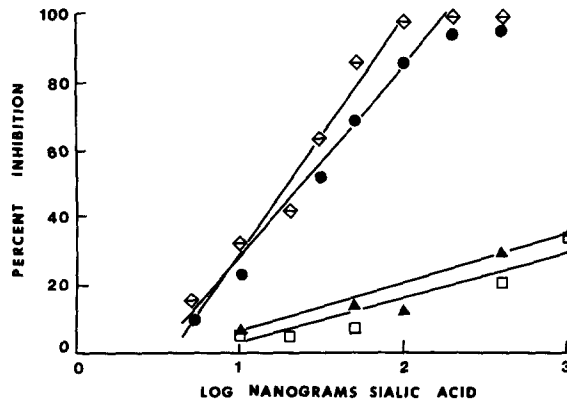FIGURE 5. Inhibition of the binding of [^{3}H]-labeled GBMP to human IgM^{NOV} using (▲) NeuNAc$_{\sim 20'}$ (□) periodate oxidized NeuNAc$_{\sim 20'}$ (◇) high m.w. GBMP and (●) NeuNAc$_{\sim 20}$-BSA conjugate.

could be due to some alteration in specificity of the labeled GBMP or a contribution to binding from the solid-phase assay.

Fig. 5 shows that $\alpha(2\to 8)$NeuNAc$_{\sim 20}$ and periodate oxidized $\alpha(2\to 8)$NeuNAc$_{20}$ are much less active than the high molecular weight GBMP and $\alpha(2\to 8)$NeuNAc$_{\sim 20}$-BSA in inhibiting binding of ^{3}H-labeled GBMP to human IgM^{NOV}, indicating that the multivalence of the oligomers of GBMP coupled to lipid in the intact GBMP or the oligomers of $\alpha(2\to 8)$NeuNAc$_{\sim 20}$ coupled at the nonreducing ends to BSA produce equivalent increased binding based on sialic acid content. It may also be concluded that the terminal nonreducing ends are not involved in the epitope.

Fig. 6 shows that the N-propionylated hexamer of $\alpha(2\to 8)$-linked NeuNAc is equivalent on a molar basis to the N-acetyl hexamer in inhibiting binding of ^{3}H-labeled GBMP to human IgM^{NOV}; the N-propionyl dimer was some what less potent than the N-acetyl dimer. The reactivity of the N-propionyl compounds may indicate that the binding of IgM^{NOV} could be directed toward the acidic side of the GBMP.

Fig. 7 shows ELISA titration of binding of IgM^{NOV} and of H46 using $\alpha(2\to 8)$NeuNAc$_{\sim 20}$-BSA and $\alpha(2\to 8)$NeuNPr$_{\sim 20}$-BSA as coating antigens. More H46 antibody than IgM^{NOV} is bound at the peak but the titers are comparable. Again N-acetyl and N-propionyl $\alpha(2\to 8)$NeuNAc$_{\sim 20}$-BSA give indistinguishable binding
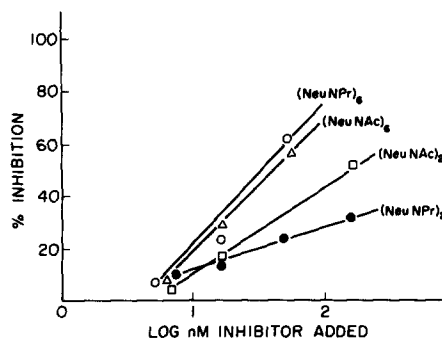

FIGURE 6. Inhibition of the binding of [^{3}H]-labeled GBMP to human IgM^{NOV} using linear $\alpha(2\to 8)$-linked N-acetylated (NeuNAc)n and the equivalent N-propionylated (NeuNPr)n oligosaccharides.
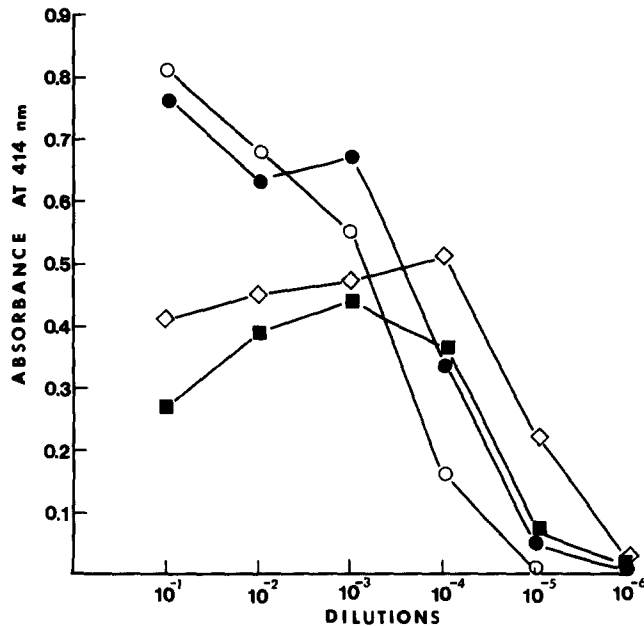
FIGURE 7. ELISA titrations of horse IgM using (●) Neu-NAc$_{\sim20}$-BSA and (O) Neu-Pro$_{\sim20}$-BSA as the coating antigens and IgM^{NOV} using (■) NeuNAc$_{\sim20}$-BSA and (◇) Neu-NPr$_{20}$-BSA as the coating antigens.

curves with IgM^{NOV} as well as with H46, which are the same within experimental error.

Fig. 8, A and B, provide comparisons of the behavior in quantitative precipitin tests of various intact HGBMPs and the Gotschlich sample lot XIX, after treatment with phospholipase C, phospholipase A2, and dilute alkali, as well as after coupling to BSA. With monoclonal IgM^{NOV} (Fig. 8 A), phospholipase C-treated lot XIX, which did not split off the lipid, shows very little change in its quantitative precipitin curve; it is slightly less active per unit weight and this could be due to slight contamination with inert materials in the phospholipase C which would reduce the amount of polysaccharide in the weighed sample. The phospholipase A2- and the alkali-treated samples showed somewhat reduced precipitating activity per unit weight but were essentially identical. Both precipitated $\sim90\%$ of the maximum N obtained with lot XIX and the other GBMP. However, the amount of polysaccharide needed to give 50% precipitation was 1.7 μg of the enzyme-treated samples as compared with 0.8 μg for the original GBMPs. The GBMP-BSA precipitated about the same amount of N at the maximum, but ~2.9 μg was needed for 50% precipitation.

With polyclonal H46, the phospholipase C-, phospholipase A2-, alkali-treated lot XIX and GBMP-BSA precipitated a maximum of 4.6, 3.7, 2.8, and 4.4 μg total N as compared with 7.2 μg N for untreated lot XIX, and considerably more of each sample was required for precipitation. Nevertheless, both IgM^{NOV} and H46 gave good precipitin curves after these various treatments, showing that the individual poly α(2→8)NeuNAc chains liberated enzymatically or by alkali are multivalent. Why precipitation of these delipidated compounds with polyclonal H46 is less than with monoclonal IgM^{NOV} is not clear.
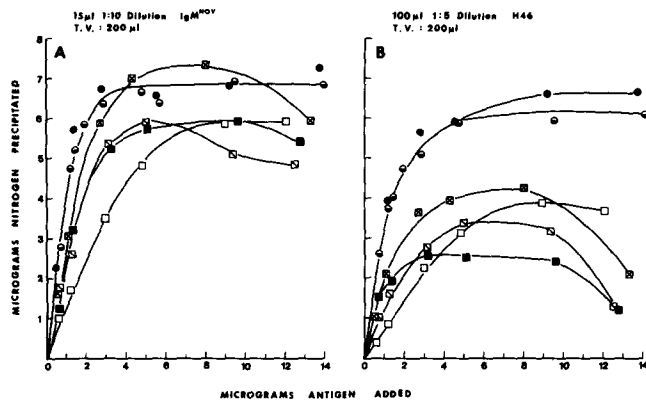
FIGURE 8. Comparison of intact GBMP lot XIX (Gotschlich) (●); (Moreno) (◓); GBMP-BSA (□); and phospholipase A2 (◩) and alkali (■) delipidated GBMP and control phospholipase C treated GBMP (⊠). Nitrogen of the antigen has been subtracted in each case. A IgM^{NOV}; B H46.

Discussion

The epitope of the group B meningococcal and of *E. coli* K1 polysaccharide is unique among polysaccharide epitopes thus far reported. Other carbohydrate epitopes which may vary in size from a di- or trisaccharide to a hexa- or heptasaccharide in site filling complementary area (24) and may be specific for the terminal nonreducing end or for internal segments of the polysaccharide (7). With the group B meningococcal epitope, increased inhibition was obtained with oligosaccharides of chain length up to 16 U with H46 (13) and up to about 12 α(2→8)linked NeuNAc residues, the longest tested with IgM^{NOV} (Figs. 3 and 4). Moreover, monoclonal IgM^{NOV} crossreacts extensively with poly(A), poly(I), and with denatured DNA whereas neither the horse polyclonal antibody, H46 (1), nor a mouse monoclonal anti-poly α(2→8)NeuNAc (16) show such crossreactivity. It is important that the inhibition of precipitation by various oligosaccharides of IgM^{NOV} by GBMP and by poly(A) showed only minor differences as discussed above (Fig. 4, *A* and *B*). By electrophoresis of IgM^{NOV}, the monoclonal spike was removed completely by group B polysaccharide and poly(A). Since they are both binding to the same sites on IgM^{NOV}, the data lend support to the hypothesis (1) that negative charges with appropriate spacing is what is being recognized (see reference 6).

The uniqueness of the epitope is strongly supported by ^{13}C-NMR spectra (15), which clearly established that in oligosaccharide (α(2→8)NeuNAc)$_{10}$ only the inner six residues assume a conformation that is representative of the epitope of GBMP. This number of residues is consistent with the size range of the usual carbohydrate epitopes (7, 9, 10, 24). The existence of such conformationally controlled epitopes (13, 25) adds another dimension to the already protean specificity of the immune system and many other instances may be found.

Gotschlich et al. (19) found that the group B polysaccharide is built of multiple chains of poly-α(2→8)NeuNAc connected by lipid; this would provide multiple epitopes specific for the non-reducing end in addition to the internal epitopes, suggesting the possibility (13, 14) that the difference between the combining sites of IgM^{NOV} and H46 might be related to this difference. Our data rule out this explanation because the single chains liberated by enzyme or alkaline hydrolysis retain their precipitating power with both IgM^{NOV} and H46 (Fig. 8). In addition, substi-

tution of BSA at the nonreducing ends of GBMP does not affect the capacity of both to precipitate or to bind in ELISA based on equivalent sialic acid content (Fig. 7), and selective periodate oxidation of the terminal nonreducing residue does not affect binding. Thus, the differences between the combining sites of IgM^{NOV} and H46 must be accounted for with both kinds of sites being complementary to oligomeric patterns of internal segments of α(2→8)linked NeuNAc not involving the terminal nonreducing end. The most important test of such a hypothesis would be its ability to account for the reactivity of IgM^{NOV} to react with poly(A), poly (I), and denatured DNA, whereas H46 (1) and the mouse monoclonal (16) do not.

It has been demonstrated (15) that in the conformationally controlled epitope of the GBMP or of the poly-α(2→8)NeuNAc)$_{\sim 20}$, the carboxylate and acetamido groups are lined up on opposite sides of the molecule. One might hypothesize that the groove-type IgM^{NOV} binds to an epitope located on the carboxylate side of the molecule. It is of interest that Zopf et al. (26) have shown that some antibodies can be directed toward one side of a tetrasaccharide while others can be specific for the opposite side, each of entirely different specificity. Consistent with this is the finding (Fig. 6) that the N-acetamido and the N-propionyl hexamers react equally well with IgM^{NOV}, although the N-acetamido dimer is somewhat better than the N-propionyl dimer. The latter compounds might be too small to react typically in a large site. If the epitope involved their direct contact in the groove, the N-propionylated compounds might be expected to react relatively poorly compared with the N-acetamido compounds. This would suggest that the original hypothesis (1) that a similar distribution of negative charges on the GBMP and the poly(A), poly(I), and denatured DNA is the basis of the crossreactivity is correct. Single-stranded poly(A), poly(I), and denatured DNA with all the phosphate groups on one side of the molecule and all the bases on the other exist and would mimic the basic structure of the conformationally controlled inner residues of the GBMP. In addition, these molecules exhibit secondary structure; poly(A) in its unprotonated form has helical and coiled domains (27). It would be of interest to determine which of these conformations contains the epitope that binds to IgM^{NOV}, since if it were the former it would provide evidence that the conformationally controlled epitope of GBMP is also helical in structure. A structure consisting of two parallel intertwined helical chains has also been proposed (28) for poly(A) fibers. At present it is not possible to account definitively for the ability of IgM^{NOV} to react with poly(A), poly(I), and denatured DNA, whereas H46 does not. Since both are conformationally controlled, IgM^{NOV} must be recognizing some oligomeric structures common to GBMP and the polynucleotides, whereas H46 is recognizing a pattern not shared by GBMP and poly(A), poly (I), poly(G), and denatured DNA. The data in Figs. 7 and 8 indicate that both are reacting with the acidic side of the molecule. It is possible that the distribution of negatively charged and protonated carboxyls and phosphates under the pH conditions used in Figs. 7 and 8 is such as to determine this. Such an instance was found in which two human monoclonals IgM^{MAY} and IgM^{WEA} both most specific for Klebsiella K30 with terminal nonreducing 3,4-pyruvylated DGal-linked α showed a striking difference in ability to crossreact with K21 with terminal nonreducing 4,6-pyruvylated DGal-linked β (29). K30 and K21 gave identical precipitin curves with IgM^{MAY} from pH 4-7 and IgM^{WEA} with K30, whereas with K21 no precipitation was obtained from pH 5.3-7 but at pH 4, K21 precipitated identically to K30 (30). These

findings suggest that a study of the precipitin curves of IgM^{NOV} and H46 at different pH with GBMP and with poly(A) might clarify the basis for this crossreactivity.

Summary

The fine structure of the combining site of human mAb IgM^{NOV} to poly-$\alpha(2\rightarrow8)$linked NeuNAc, the epitope of the group B meningococcal and *E. coli* K1 polysaccharides, has been probed using RIA and ELISA. Inhibition by oligomers ranging from 2 to 12 residues was used to assay binding to IgM^{NOV} by group B meningococcal polysaccharide preparations (GBMP) or by poly(A). The inhibitory properties of the oligomers were almost identical in both assays of the binding of GBMP to horse IgM (H46). This evidence and the finding that both GBMP and poly(A) precipitated IgM^{NOV} equally per unit weight indicated that the epitope of poly(A) must mimic an equivalent epitope on GBMP despite the absence of any apparent common structural features in the two molecules. Unlike most carbohydrate–anticarbohydrate systems in which the site is saturated by oligomers of up to six or seven sugars, all the anti-$\alpha(2\rightarrow8)$NeuNAc systems above required much larger oligomers. Because these oligomers are larger than the maximum size of an antibody site the epitope must be conformationally controlled, and this has been confirmed by nuclear magnetic resonance spectroscopy.

However, despite the above similarities, GBMP and poly(A) were differentiated in that only GBMP bound to H46. Smaller linear molecules obtained by delipidating the GBMP, as well as periodate-oxidized GBMP with its nonreducing end oxidized or linked covalently to BSA, bound to and precipitated IgM^{NOV} and H46. This showed that, despite their differences, terminal nonreducing ends were not involved and that both epitopes were located in the conformationally controlled inner residues of the GBMP. The difference thus must reside in the ability of IgM^{NOV} and H46 to recognize different structural aspects of the same conformationally controlled inner residues. The ELISA data indicate that both IgM^{NOV} and H46 have groove-type sites that bind exclusively to an epitope located on the acidic side of the inner residues. The differences determining the ability of IgM^{NOV} and the failure of H46 to cross-react with poly(A), poly(I), and denatured DNA, may depend on differences in the degree of protonation required by each antibody, and this may be clarified by a study of the effects of pH on the precipitin behavior of IgM^{NOV} and H46.

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