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Epitopes Recognized by a Nonautoreactive Murine Anti-*N*-Propionyl Meningococcal Group B Polysaccharide Monoclonal Antibody

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The capsular polysaccharide of *Neisseria meningitidis* group B (MBPS) is a polymer of alpha (2→8) *N*-acetyl neuraminic acid. The polysaccharide is chemically identical to an autoantigen, polysialic acid (PSA), and is a poor immunogen, even when conjugated to protein carriers. Immunization of mice with MBPS-protein conjugate vaccines, in which *N*-acetyl groups have been replaced by propionyl groups (*N*-Pr MBPS), elicits serum bactericidal antibodies. A subpopulation of these antibodies do not cross-react with human PSA. The reasons for the increased immunogenicity of *N*-Pr MBPS and the antigenic targets of the bactericidal nonautoreactive antibodies are unknown. In this study, we investigated the antigenic targets of a protective murine monoclonal antibody (MAb) prepared against a *N*-Pr MBPS-tetanus toxoid conjugate vaccine. Binding of the MAb to *N*-Pr MBPS (as demonstrated by an enzyme-linked immunosorbent assay) and bactericidal activity were inhibited by de-*N*-acetylated MBPS and re-*N*-acetylated MBPS, which indicate that *N*-propionyl groups are not obligatory determinants for binding. The results of affinity selection from a preparation of *N*-Pr MBPS and matrix-assisted laser desorption ionization-time of flight mass spectroscopic analysis indicated that the minimal epitope recognized by the MAb is a MBPS disaccharide containing one de-*N*-acetylated residue. Thus, the bacterial capsular epitope recognized by this bactericidal, nonautoreactive, anti-group-B MAb likely contains de-*N*-acetyl residues.

Meningitis and bacteremia caused by *Neisseria meningitidis* are serious worldwide health problems (37). To date, 12 meningococcal capsular groups defined by structurally and antigenically distinctive polysaccharide capsules have been identified (34). Group A strains cause large meningococcal epidemics in sub-Saharan Africa, while most disease in Europe and North America is caused by strains with other capsular groups (predominantly group B, C, or Y) (37).

Safe and effective capsular-based vaccines have been developed for groups A, C, Y, and W-135 but not for group B (21, 31). The group B capsular polysaccharide (MBPS), alpha (2→8) *N*-acetyl neuraminic acid, is chemically identical to polysialic acid (PSA) that is abundantly expressed by fetal tissues, including brain, heart, and kidney (8). The group B capsular polysaccharide is poorly immunogenic even when conjugated to a carrier protein, possibly as a result of immune tolerance (18). However, monoclonal antibodies (MAbs) have been prepared by immunizing mice with killed group B bacteria (25, 30, 39) or by immunizing New Zealand Black mice with plain polysaccharide or bacteria (9, 16). Human MBPS-reactive MAbs or paraproteins have also been described (2, 24, 35). These antibodies confer passive protection against meningococcal bacteremia in infant rats and elicit complement mediated bactericidal activity in vitro (24), the serologic hallmark of protective meningococcal immunity in humans (11).

In the 1980s, Jennings and coworkers investigated the immunogenicity of various derivatives of MBPS (1, 17–20, 33). Conjugate vaccines containing polysaccharides in which *N*-acetyl groups of MBPS were replaced with a variety of more hydrophobic acyl groups, particularly *N*-propionyl (*N*-Pr), were found to be immunogenic in mice and elicited protective serum bactericidal antibodies. The epitope(s) on the bacterial surface recognized by the protective anti-*N*-Pr MBPS antibodies is not known.

In a previous study, we immunized mice with *N*-Pr MBPS conjugated to tetanus toxoid and prepared a panel of anti-*N*-Pr MBPS MAbs (12). A number of the MAbs elicited complement-mediated bactericidal activity in vitro and conferred passive protection against bacteremia in an infant rat model. Of the set of protective MAbs, several showed minimal or no detectable autoreactivity with host PSA, as measured by flow cytometry studies with the human cell line CHP-134 (12), which expresses long-chain PSA (22). In subsequent studies, these MAbs also did not react by immunohistology with human fetal tissues (unpublished data).

The procedures used in the synthesis of *N*-Pr MBPS have the potential for producing unintended derivatives of MBPS. Examples include de-*N*-acylated amino groups, formation of imines with de-*N*-acylated amino groups, formation of lactones, *O*-acylation, oxidation, formation of cyclic sialic acid (27), and reduction of internal imines to secondary amines. In this study, we investigated the hypothesis that the chemical procedures used to prepare *N*-Pr MBPS resulted in unintended MBPS derivatives that coincidentally also exist in the group B capsule but not in human PSA and that these by-products were responsible, at least in part, for the protective nonautoreactive antibodies elicited by *N*-Pr MPBS conjugate vaccines.

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MATERIALS AND METHODS

MAbs. The anti-*N*-Pr MBPS murine MAb SEAM 3 (immunoglobulin G2b [IgG2b]) has been previously described (12). As shown in Fig. 1A, the MAb is bactericidal in the presence of complement. The concentration of MAb required

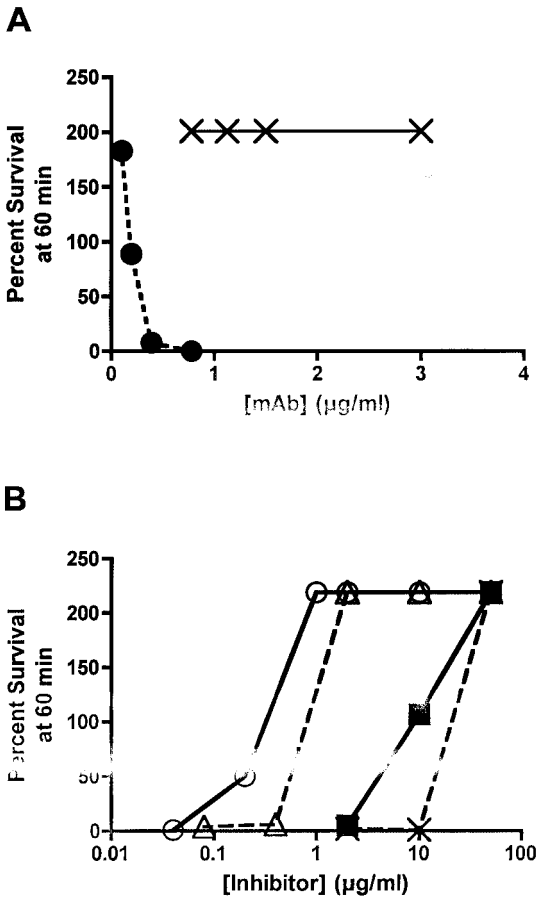

FIG. 1. (A) Dose-response bactericidal activity of the anti-*N*-Pr MBPS MAb SEAM 3 (●) compared to an irrelevant control MAb (X). The percent survival of group B strain 8047 was calculated from the CFU/ml at 60 min compared to that that of control CFU per milliliter at time zero. Note that in the presence of SEAM 3 alone, complement alone, or complement and an irrelevant MAb, there was an increase in the number of CFU per milliliter during the 1-h incubation. (B) Inhibition of anti-*N*-Pr MBPS MAb SEAM 3 bactericidal activity by different MBPS derivatives. The reaction mixture, consisting of SEAM 3, complement, and bacteria, was incubated at 37°C for 60 min in the presence of different concentrations of soluble inhibitors. The inhibitors tested were *N*-Pr MBPS (○ with solid line), re-*N*-acetylated MBPS (△ with dashed line), de-*N*-acetylated MBPS (■ with solid line), and native N-acetyl MBPS prepared from *N. meningitidis* group B (× with dot-dash line).

for 50% killing of a representative encapsulated *N. meningitidis* group B strain (8047) is 0.2 μg/ml. By flow cytometry, SEAM 3 had no detectable autoreactive activity when tested at 100 μg/ml. In multiple experiments, SEAM 3 has been shown to confer passive protection against meningococcal bacteremia in infant rats challenged intraperitoneally with *N. meningitidis* group B strain 8047, BZ232, M986, or 2996 (28, 29, 43). The anti-MBPS MAb, 2-1-B (IgM) (25) was a gift of Wendell Zollinger, Walter Reed Army Institute of Research, Silver Spring, Md., and the anti-porin P1.2 MAb was purchased from the National Institute of Biological Standards and Control, Potters Bar, Hertfordshire, United Kingdom.

MBPS derivatives. Colominic acid (i.e., *Escherichia coli* K1 capsular polysaccharide; Sigma-Aldrich, St. Louis, Mo.) was used to prepare deacetylated MBPS, employing procedures described by Guo and Jennings (13). Colominic acid is commercially available and is chemically and immunologically identical to MBPS (3, 36) except that some *E. coli* strains express C_7 or C_9 *O*-acetylated polysaccharide (32). For convenience, we refer to the colominc acid derivatives as MBPS derivatives, since after de-*N*-acetylation there also is elimination of *O*-acetylation; thus, there are no structural differences between the two polysaccharides after treatment. The only modification from the Guo and Jennings protocol is that a 2.4- by 25-cm Toyoperl HW-55F (Supelco, Bellafonte, Pa.) column was used for size exclusion chromotography instead of a Bio-Gel A 0.5 column. Also, after de-*N*-acetylation, we re-*N*-acetylated MBPS with acetic anhydride (Sigma-Adrich), which was not included in the derivatives previously prepared by Guo and Jennings. As described in Results, in some experiments, native MBPS derived from *N. meningitidis* group B strain MC58 was used for inhibition studies (referred to below as native MBPS to distinguish this polysaccharide from colominic acid). The native group B polysaccharide was isolated from culture supernatants as described by Costantino et al. (6). All of the polysaccharide derivatives had an apparent mass of >10 kDa and an average apparent mass of 30 kDa, as determined by size exclusion chromotography calibrated with dextran and protein standards. In addition, the derivatives did not contain small (degree of polymerization [Dp] < 10) oligosaccharides as determined by analytical ion exchange chromatography (Q Sepharose FF; Amersham Biosciences, Piscataway, N.J.) performed as described by Troy et al. (41).

Primary amine determination. The concentrations of free amino groups on polysaccharide derivatives were determined by using a fluorescamine (42) assay as follows. Up to 100 μl of MBPS derivative in PBS was combined with 900 μl of PBS in a 13- by 100-mm clean glass tube. While the sample was vortexed, 500 μl of fluorescamine (100 μg/ml) in acetone was added. The samples were read immediately with an LS 50B luminescence spectrometer (Perkin-Elmer, Norwalk, Conn.) with excitation at 390 nm and emission at 475 nm. Mannosamine (Sigma-Aldrich) was used as a standard.

Inhibition ELISA and bactericidal assay. Polysaccharide concentrations were determined by using the method of Svennerholm (40). Inhibition of MAb binding to *N*-Pr MBPS in an enzyme-linked immunosorbent assay (ELISA) was performed as described by Granoff et al. (12). Complement-mediated bactericidal activity was measured with *N. meningitidis* group B strain 8047 as described previously (12). In brief, the test organism was grown at 37°C for approximately 2 h in Mueller-Hinton broth supplemented with 0.25% glucose to an A_{620} value of ~0.6. After the bacteria were washed in Gey's buffer containing 1% (wt/vol) bovine serum albumin, approximately 300 to 400 CFU were added to the reaction mixture. The final reaction mixture of 40 μl contained 20% (vol/vol) complement, different concentrations of inhibitor (see Results), and a fixed concentration of MAb in Gey's buffer (10 μg/ml) that in the absence of inhibitor resulted in 0% survival of the bacteria after a 60-min incubation in the reaction mixture compared to that of control CFU per ml at time zero. The inhibitor and MAb were combined first, and then the bacteria and complement were added. Typically, bacteria incubated with the negative control antibody and complement showed a 150 to 200% increase in the number of CFU per milliliter during the 60 min of incubation (Fig. 1A). The complement source was pooled serum from infant rabbits (Cedar Lane, Hornby, Ontario, Canada). The complement source had no detectable intrinsic bactericidal activity when tested at 20 or 40% (i.e., twofold higher than the concentration used to measure bactericidal activity in the test antisera).

Affinity purification and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectroscopy of MBPS derivatives recognized by SEAM 3. MAb SEAM 3 or an irrelevant control MAb (5 μg) were incubated for 1 h at ambient temperature with 200 μl of goat anti-mouse IgG magnetic beads (Pierce Endogen, Rockford, Ill.) in phosphate-buffered saline (PBS) buffer. Sterile, silanized 0.6-ml tubes (Fisher Scientific) were used in all procedures, The beads were then washed three times with wash buffer (PBS containing 0.1% [wt/vol] Tween-20 and 0.1% [wt/vol] sodium azide). The bound MAbs were cross-linked to the beads by resuspending the bead-antibody complex in PBS buffer containing 1 mM BS^3 (Pierce Endogen) and vortexing for 30 min at ambient temperature. Tris buffer was added to a final concentration of 0.1 M (pH 8.0) for 10 min, followed by washing the beads three times with PBS. The cross-linked MAb beads were stored at 4°C in PBS until used. *N*-Pr MBPS (100 μg) in 200 μl of PBS buffer was added to the magnetic beads containing the cross-linked MAbs. The mixture was mixed on a vertically rotating platform for 1 h at ambient temperature. The unbound material was removed, and the beads were washed five times with wash buffer. After the last wash, the beads were resuspended in 200 μl of PBS buffer containing 0.001 U of exosialidase (EC 3.2.1.18; Sigma-Aldrich, St. Louis, Mo.) and incubated with gentle mixing overnight at 37°C. After the supernatant was removed, the beads were washed five times with 25 mM ammonium carbonate buffer (pH 8.0). Bound MBPS derivatives were eluted with 0.1 M triethylamine (Sigma-Aldrich).

A matrix solution of 50% acetonitrile-water saturated with 2,4,6-trihydroxy acetophenone (THAP; Fluka, Ronkonkoma, N.Y.) was spotted (0.5 μl) onto the target plate. After drying the solution eluted from the beads in a spin-vac (Savant), the residue was resuspended in 4 μl of water, and three 0.5-μl aliquots of the sample were spotted on top of the dried matrix spot. MALDI-TOF mass

TABLE 1. Inhibition of binding of the anti-*N*-Pr MBPS mAb SEAM 3 to *N*-Pr MBPS by ELISA

Inhibitor	Inhibitor $concn_{50\%}$[a]
N-acetyl MBPS[b]	>100
Colominic acid[c]	>100
De-*N*-acetyl MBPS	0.8
Resynthesized *N*-acetyl MBPS[d]	0.32
Synthesized *N*-Pr MBPS[e]	0.004

[a] Concentration in micrograms per milliliter inhibiting 50% of maximum optical density at 405 nm after 30-min incubation with substrate. In the absence of inhibitor, the optical density was ~1.

[b] Isolated from *N. meningtidis* group B bacteria. In an ELISA, both MBPS and colominic acid inhibit binding of anti-MBPS MAb 2-1-B to *N*-acetyl MBPS at competitor concentrations of <0.1 μg/ml (data not shown).

[c] Prepared from *E. coli* K1. Colominic acid is chemically and immunologically identical to MBPS (3, 36), except that some *E. coli* K1 strains express C_7 or C_9 *O*-acetylated polysaccharide (32).

[d] Resynthesized N-Ac MBPS that has been de-acetylated and re-N-acetylated as described in Materials and Methods.

[e] N-Pr MBPS that has been de-acetylated and *N*-propionylated as described in Materials and Methods.

spectroscopy (Autoflex, Bruker Daltonics, Billerica, Mass.) was performed in the negative reflector mode (30 shots of an N_2 laser). The mass spectrum was calibrated by external standards (Bruker Daltonics). The error of the observed masses was estimated to be ≤0.1%. The THAP matrix reacts with free amines to form imine derivatives. In initial experiments, we observed the formation of small amounts of THAP derivatives of the major observed ions, suggesting that the ions contained reactive amino groups. To drive the formation of THAP imine derivatives, the sample-matrix mixture spotted on the plate was incubated under vacuum for >2 days to remove water and was then reanalyzed by MALDI-TOF.

RESULTS

Inhibition of anti-*N*-Pr MBPS binding by MBPS derivatives. The procedures used in the synthesis of *N*-Pr MBPS have the potential for producing unintended derivatives of MBPS. To evaluate whether MAbs prepared to *N*-Pr MBPS are reactive with side products produced during the synthesis of *N*-Pr MBPS, we prepared both *N*-Pr and re-*N*-acetylated MBPS. Table 1 summarizes the ability of the respective derivatives to inhibit the binding of the anti-*N*-Pr MBPS MAb to *N*-Pr MBPS in an ELISA. In control experiments, soluble colominic acid or native MBPS prepared from an *N. meningitidis* group B strain completely inhibited binding in an ELISA of the known autoreactive anti-MBPS MAb 2-1-B (class IgM) (25) to *N*-acetyl MBPS at concentrations of the competitor of less than 0.1 μg/ml (data not shown). Colominic acid or MBPS did not inhibit binding of the anti-*N*-Pr MBPS MAb. In contrast, low concentrations of *N*-Pr MBPS were highly effective inhibitors (≥0.1 μg/ml) (Table 1). These results were expected, since the MAb was selected for the ability to bind to *N*-Pr MBPS in an ELISA but not to MBPS (as an adipic dihydrazide derivative) (12). De-*N*-acetylated MBPS inhibited binding of SEAM 3 to *N*-Pr MBPS. This result suggests that the anti-*N*-Pr MBPS MAb recognizes epitopes that, in part, contain de-*N*-acylated residues. Re-*N*-acetylated MBPS prepared by acetylating de-*N*-acetyl MBPS with acetic anhydride was only a slightly better inhibitor of the MAb than the de-*N*-acetyl MBPS derivative. These results show SEAM 3 recognizes epitopes that do not contain propionyl groups. Also, the epitopes recognized appear to result from the de-*N*-acetylation and reacylation procedures.

N-Pr MBPS is not known to exist in the capsule of group B meningococcus, Therefore, either the *N*-Pr derivative mimics a conformational epitope present in the capsule, as suggested by Jennings and coworkers (4, 7, 23, 33), or the intended or unintended MBPS derivatives that are produced during the chemical procedures used to prepare *N*-Pr MBPS elicit antibodies that react with unrecognized modified PS epitopes present on the native bacterial capsule. Figure 1B summarizes data on the ability of different MBPS derivatives to inhibit bactericidal activity of the anti-*N*-*N*-Pr MBPS MAb against *N. meningitidis* group B strain 8047. Re-*N*-acetylated MBPS was nearly as good an inhibitor as *N*-Pr MBPS (0.6 μg/ml compared to 0.2 μg/ml) whereas 7-fold-higher concentrations of de-*N*-acetyl MBPS and >20-fold-higher concentrations of MBPS prepared from group B bacteria were required for inhibition. There was no significant inhibition of bactericidal activity by a concentration of 50 μg/ml of an irrelevant meningococcal capsular polysaccharide (group A). Thus, the capsular epitope recognized by SEAM 3, a bactericidal and protective but nonautoreactive anti-*N*-Pr MBPS MAb, is present in intermediates produced during the synthesis of the *N*-Pr MBPS derivative that do not contain propionyl groups. Although not shown, a concentration of <0.1 μg/ml of *N*-acetyl MBPS inhibited bactericidal activity of the control MAb 2-1-B, directed against *N*-acetyl MBPS.

Since SEAM 3 recognizes, at least in part, MBPS epitopes that contain de-*N*-acetyl residues, we attempted to quantify the amount of de-*N*-acetyl residues in the various MBPS derivative preparations by measuring the amount of primary amine using two methods: 2,4,6 trinitrobenzene sulfonic acid (14) and fluorescamine (42). Surprisingly, both methods proved insensitive in detecting primary amines in control de-*N*-acetylated MBPS preparations, whereas both reagents detected primary amines in the mannosamine standard. The results suggest that the amino groups of de-*N*-acetyl MBPS are less reactive than primary amines in other amino sugars. Nevertheless, the *N*-Pr MBPS polysaccharide preparation used in this study was found to contain 0.5% free primary amine by the fluorescamine assay. There was no fluorescamine-reactive free amine detected in the preparation of resynthesized *N*-acetyl MBPS. Both measurements are likely to be underestimates of the amount of de-*N*-acetylated polysaccharide present (see below).

Affinity selection and MALDI-TOF analysis of MBPS derivatives recognized by SEAM 3. The MBPS derivatives in a preparation of *N*-Pr MBPS that are recognized by SEAM 3 were purified by incubating the polysaccharide preparation with magnetic beads containing SEAM 3 that had been cross-linked to goat anti-mouse IgG, prepared as described in Materials and Methods. Since the bound polysaccharides were too heterogeneous to be analyzed by mass spectroscopy, the antibody/polysaccharide complex was treated with exosialidase to decrease the size of the polysaccharide to that protected from digestion by antibody binding. The MALDI-TOF mass spectrum of the material eluted from SEAM 3 after sialidase treatment is shown in Fig. 2. There were no ions observed for similar experiments performed with control MAbs. None of the observed ions corresponded to MBPS derivatives containing propionyl groups. However, the major ion at m/z = 557.32 Da was consistent with an *N*-acetyl MBPS disaccharide in which one residue was de-*N*-acetylated (Fig. 3). Nearly all of

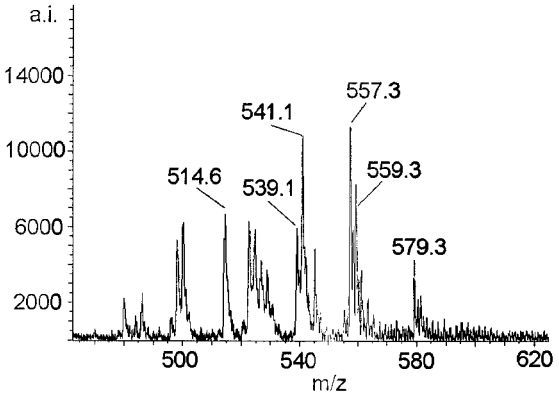

FIG. 2. A portion of the MALDI-TOF mass spectrum of MBPS derivatives selected by SEAM 3.

TABLE 2. Summary of mass spectroscopy data for MBPS derivatives selected by SEAM 3 from a preparation of *N*-Pr MBPS

Ion[a]	Calculated mass		Observed *m/z*
	Mono[c]	Average	
$[M-H]^-$	557.18	557.48	557.32
$[M-2H+Na]^-$	579.16	579.46	579.34
$[M-H+2H]^{-b}$	559.20	559.50	559.40
$[M-H+2H-H_2O]^{-b}$	541.17	541.45	541.12
$[M-H-H_2O]^-$	539.17	539.46	539.25
$[M-H-Ac]^-$	515.17	515.44	514.58

[a] $[M-H]^-$ is the de-*N*-acetyl MBPS disaccharide shown in Fig. 3. Ac, acetyl.
[b] Derivative may result from reduction of the reducing end ketone by sodium borohydride present during the de-*N*-acetylation reaction.
[c] Mono, monoisotopic.

the other ions were related to the 557.32 ion as shown in Table 2. These other ions included the sodium salt, reduced (presumably during the de-*N*-acetylation reaction) derivatives, and derivatives of the putative disaccharide or reduced disaccharide in which water, formaldehyde, and a second acetyl group, respectively, were removed. All are consistent with modifications of the putative disaccharide shown in Fig. 3 that could occur during the synthetic procedures. In addition, the *m/z* 557.3 and 539.2 ions form imine derivatives with the THAP (*m/z* = 707.2 and 689.3, respectively) (data not shown). The latter observation shows the presence of a free amine in both compounds since the reaction with the matrix is amine specific.

DISCUSSION

Since the original description of *N*-Pr MBPS conjugate vaccines by Jennings and coworkers, the ability of *N*-Pr MBPS protein conjugate vaccines to elicit high titers of serum bactericidal antibody has been confirmed by other investigators in studies conducted with mice (12) and subhuman primates (10). Although a subset of the bactericidal antibodies elicited in these studies was found to be autoreactive with host PSA, the majority of the bactericidal antibodies did not react with host PSA. It is not understood why replacing *N*-acetyl groups of MBPS with *N*-propionyl groups and conjugating the resulting chemically modified polysaccharide to a protein carrier increases immunogenicity and elicits antibodies that can bind with the bacterial capsular polysaccharide but not with host PSA. A better understanding of the *N*-Pr MBPS epitopes responsible for eliciting protective antibodies might permit design of more optimally immunogenic vaccines capable of eliciting protective antibodies in humans (5), while avoiding some of the safety concerns of autoreactivity to host PSA.

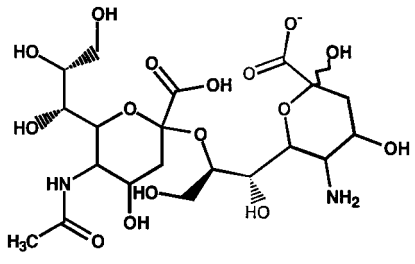

FIG. 3. Putative structure of a MBPS disaccharide selected by anti-*N*-Pr MBPS MAb SEAM 3. The derivative was selected by SEAM 3 from a preparation of *N*-Pr MBPS and was protected from exosialidase digestion by antibody binding. Since the actual structure has not been determined, the *N*-acetyl-containing residue can be located at either the reducing or nonreducing end of the disaccharide.

Based on the studies of the cross-reactivity of human monoclonal macroglobulin IgM^{NOV} with MBPS and poly(A), absorption studies with MBPS derivatives, nuclear magnetic resonance (NMR) studies of MBPS, and x-ray crystallographic studies of an anti-MBPS MAb, Jennings and coworkers suggested that propionylation of MBPS mimics a complex extended helical epitope of native MBPS. They proposed that this structure, in association with a second hypothetical surface-accessible molecule that is present on the group B capsule but not in host tissues may be the epitope recognized by non-autoreactive anti-*N*-Pr MBPS Abs (7, 26, 33). Consistent with this hypothesis was their observation that bactericidal activity and lack of autoreactivity of certain anti-*N*-Pr MBPS MAbs correlated with antibody binding to a high-molecular-mass polysaccharide (≥11 kDa) (33), which the authors suggested would more likely contain the putative helical structure (17, 33, 23). However, the results of recent computational and ^1H and ^{13}C NMR studies of MBPS (15) contradict conclusions of earlier NMR studies (15, 40, 42–45) and suggest that MBPS is a "random coil chain in solution, and therefore, does not have antigenic epitopes dependent upon a rigid, ordered conformation" (15).

Furthermore, using a similarly modified *N*-Pr MBPS-protein conjugate vaccine, we prepared a panel of bactericidal anti-*N*-Pr MBPS MAbs (12). The antigenic specificities of some of our anti-*N*-Pr MBPS MAbs were different than those described by Jennings and coworkers (33). For example, whereas their bactericidal anti-*N*-Pr MBPS MAb recognized a minimal *N*-Pr MBPS epitope of Dp ≥ 12 (23), binding of several of our bactericidal MAbs to *N*-Pr MBPS was inhibited by oligosaccharides with Dp < 7 (average Dp, 3.8) (12), a size too small to adopt a stable conformational structure. One example of such an antibody is SEAM 3, which was used in the present study. We have previously shown that the anti-*N*-Pr MBPS MAbs preferentially recognize capsular epitopes of *N. meningitidis* group B bacteria compared with those of host PSA (12). In the present study, we found that the bactericidal activity of

SEAM 3, which lacks detectable autoreactivity, was inhibited by low concentrations of resynthesized *N*-acetyl MBPS (Fig. 1B). Therefore, the epitope recognized by this MAb clearly does not depend on the presence of the *N*-Pr groups. In addition, we have shown that SEAM 3 selectively binds a molecule having a mass corresponding to a MBPS disaccharide in which one residue is de-*N*-acetylated. Taken together, the results indicate that the epitope recognized by SEAM 3 contains de-*N*-acetyl sialic acid residues. Importantly, the polysaccharide component of the *N*-Pr MBPS-tetanus toxoid conjugate vaccine used to prepare our anti-*N*-Pr MBPS MAbs (12) was 84% propionylated as determined by ^{1}H NMR, even though the de-*N*-acetylated MBPS was treated with a severalfold excess of propionic anhydride (38). Thus, at least 15% of the residues contained amino groups that were either unreactive or were otherwise modified.

As noted above, MAb SEAM 3 lacks autoreactivity with host PSA and is bactericidal (12) and highly protective in the infant rat group B meningococcal bacteremia model (28). Thus, the polysaccharide structure recognized by this MAb could form the basis of a safe and protective group B vaccine.

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