

**N-PROPIONYLATED GROUP B MENINGOCOCCAL  
POLYSACCHARIDE MIMICS A UNIQUE EPITOPE ON  
GROUP B *NEISSERIA MENINGITIDIS***

BY HAROLD J. JENNINGS, ANDRZEJ GAMIAN, AND FRASER E. ASHTON\*

*From the Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6; and the \*Bureau of Microbiology, Laboratory Center for Disease Control, Ottawa, Ontario, Canada 0L2*

Group B meningitis remains a major world health problem and the poor immunogenicity (1) of the group B meningococcal polysaccharide (GBMP) prevents the formulation of a comprehensive polysaccharide vaccine against meningococcal meningitis (2, 3). Recently, a highly immunogenic form of the GBMP was made by conjugating a chemically modified GBMP to tetanus toxoid (TT) (4). In this modification, *N*-propionyl (*N*-Pr) groups were substituted for the *N*-acetyl groups of the GBMP. The antibodies induced in mice by the *N*-Pr-GBMP-TT conjugate were bactericidal for GBM organisms, and on this evidence it must be considered as a potential vaccine. However, it has been inferred that such a vaccine could only be successful at the risk of breaking tolerance, and this hypothesis is legitimized by the identification of  $\alpha$ -2 $\rightarrow$ 8-linked oligomers of sialic acid common to both the GBMP (5, 6) and to human and animal tissue (7, 8). Therefore, it was important to identify the epitope on the *N*-Pr-GBMP-conjugate associated with the production of bactericidal antibody. The conjugate induced in mice two distinct populations of *N*-Pr-GBMP-specific antibodies, associated with two different epitopes, one which is shared by the GBMP. However, of particular interest to the above problem is the fact that bactericidal activity was associated with an epitope on the *N*-Pr-GBMP not present on the exogenous GBMP.

#### Materials and Methods

**Materials.** The strains (groups and protein serotypes in parenthesis) of *Neisseria meningitidis* disease isolates used in this study were M986 (B:2a:P1.2), 2996 (B:2b:P1.2), 80278 (B:2b:P1.2), 83101 (B:2b:P1.2), 85066 (B:2b:P1.2), 44/76 (B:15:P1.16), 85095 (B:nontypable), 81014 (C:2a:P1.2), 85088 (W135:nontypable), and 85079 (A). The high molecular weight GBMP was provided by Dr. W. Hankins, Connaught Laboratories, Swiftwater, PA. The *N*-propionylation of the GBMP and the subsequent synthesis of the *N*-Pr-GBMP-TT conjugate have been previously described (4). Antisera were obtained from two different groups of female CF1 mice after threefold intraperitoneal injections with the *N*-Pr-GBMP-TT conjugate in CFA (4), and the antisera were pooled (pools 1 and 2) and heat inactivated at 56°C for 30 min.

**Bactericidal Assays.** A modification of a microbactericidal assay (9) was used. Twofold dilutions of the serum were made in Dulbecco's PBS, pH 7.2, containing magnesium and

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TABLE I  
*Bactericidal Activity of Pool 1 of Mouse Anti-N-Pr-GBMP-TT  
 Conjugate Serum against Meningococcal Disease Isolates*

Strain (serogroup/serotype)	Bactericidal titer
M986 (B:2a:P1.2)	4,096
2996 (B:2b:P1.2)	2,048
80278 (B:2b:P1.2)	4,096
83101 (B:2b:P1.2)	2,048
85066 (B:2b:P1.2)	1,024
44/76 (B:15:P1.16)	4,096
85095 (B:nontypable)	4,096
81014 (C:2a:P1.2)	<4
85088 (W135:nontypable)	<4
85079 (A)	<4

calcium (10) and 0.1% gelatin (DPBG) so that 50  $\mu$ l of serum dilution were present in each well of a sterile tissue culture multiwell plate (Linbro Chemical Co., Hamden, CT). 20- $\mu$ l of pooled undiluted baby rabbit serum was added to each well as a source of complement. Controls included 50  $\mu$ l DPBG and 20  $\mu$ l of complement, 70  $\mu$ l of DPBG, and 50  $\mu$ l of serum dilution plus 20  $\mu$ l of DPBG to ensure that activity was not due to agglutination of the organisms. Meningococcal strains used in the assay were grown on gonococcal (GC) medium (11) at 36°C in an atmosphere of 5% CO<sub>2</sub> for 4–5 h. The organisms were diluted in DPBG and 30  $\mu$ l of suspension containing ~100 CFUs were added to each well. The tissue culture plates were incubated at 36°C for 30 min after which time 50  $\mu$ l were removed from each well and spread on GC medium. The plates were incubated at 36°C in an atmosphere of 5% CO<sub>2</sub> for 24 h and the number of colonies were counted. The highest serum dilution (expressed as the reciprocal) causing a  $\geq$ 90% reduction in viable count was considered the bactericidal titer of the serum. The results were reproducible within one serum dilution.

*Quantitative Microprecipitin Analyses.* Analyses were performed in duplicate essentially by the method of Kabat and Mayer (12). 20- $\mu$ l aliquots of pool 1 antiserum were diluted with 80  $\mu$ l of physiological-buffered saline (PBS, 0.01 M) at pH 7.2 and were reacted with increasing concentrations of polysaccharides in a total volume of 200  $\mu$ l (adjusted with PBS). The tubes were incubated for 1 h at 37°C and for 3 d at 4°C, centrifuged, washed, and the quantity of protein in the pellet was determined by the method of Lowry et al. (13). For absorption experiments the pooled serum (300  $\mu$ l) was incubated with 30  $\mu$ g of N-Pr-GBMP or 150  $\mu$ g of GBMP (equilibrium quantities) in 150  $\mu$ l of PBS for 1 h at 37°C followed by 4 d at 4°C. The precipitates were removed by centrifugation and the removal of essentially all GBMP-specific antibodies from the GBMP-absorbed serum was monitored by RIA using <sup>3</sup>H-labeled GBMP (6). Precipitin experiments on the absorbed serum were carried out as described above using PBS to adjust to the dilution used previously. All the above experiments were repeated on another preparation of mouse anti-N-Pr-GBMP-TT conjugate serum (pool 2).

### Results and Discussion

Because the N-Pr-GBMP-TT conjugate can induce high titers of GBMP-specific IgG antibodies in mice (4), it must be considered as a vaccine against meningitis caused by group B *N. meningitidis*. Additional evidence that supports its candidacy is that antibodies raised in mice by the N-Pr-GBMP-TT conjugate are highly bactericidal for group B meningococcal organisms. Experiments to confirm this are shown in Table I and indicate that the pooled mouse antiserum is bactericidal for a number of group B meningococcal disease strains isolated in diverse geographical areas. The specificity of this bactericidal activity is demonstrated by the inability of the pooled mouse antiserum to exhibit any significant

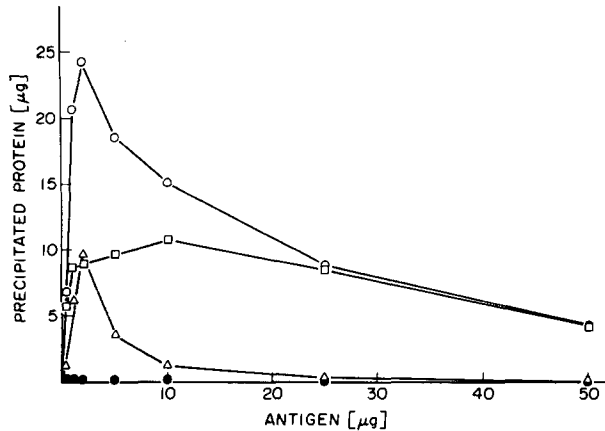


FIGURE 1. Quantitative micro-precipitin curves of mouse anti-*N*-Pr-GBMP-TT conjugate serum (pool 1) with *N*-Pr-GBMP (○) and GBMP (□). Also, the same serum was absorbed with the GBMP before precipitation with *N*-Pr-GBMP (Δ) and GBMP (●).

TABLE II  
Bactericidal Activity of Nonabsorbed and Absorbed Pooled Mouse  
Anti-*N*-Pr-GBMP-TT Conjugate Sera

Absorbing antigen	Bactericidal Titer	
	Pool 1	Pool 2
None	2,048	512
GBMP	1,024	512
<i>N</i> -Pr-GBMP	<16	<4

Meningococcal strain M986 was used in these assays.

bactericidal activity against *N. meningitidis* groups A, C, and W-135 organisms. These experiments also demonstrate that the bactericidal activity of the mouse *N*-Pr-GBMP-TT conjugate antiserum is completely independent of protein serotype, and on this evidence it seems reasonable to assume that the bactericidal activity is due to crossreactive GBMP-specific antibodies. However, this was not substantiated in subsequent experiments.

The presence of two distinct populations of antibodies in the pooled mouse *N*-Pr-GBMP-TT conjugate antiserum was demonstrated in the quantitative precipitin experiments shown in Fig. 1. While the homologous *N*-Pr-GBMP precipitated ~1.25 mg/ml of antibody from the antiserum, the heterologous GBMP was also able to precipitate approximately half this amount. Furthermore, after absorption of the antisera with the GBMP the *N*-Pr-GBMP was still able to precipitate substantial amounts of the antibody. These experiments confirm that the *N*-Pr-GBMP-TT conjugate antiserum contains two populations of *N*-Pr-GBMP specific antibodies, based on two different epitopes, only one of which is crossreactive with the GBMP.

Using the above absorbed antisera it was also possible to determine which of the above epitopes was responsible for the induction of bactericidal antibodies in the mice (Table II). Absorption of two different batches of pooled mouse *N*-Pr-GBMP-TT antisera with the *N*-Pr-GBMP removed virtually all of the bactericidal activity against group B meningococcal organisms in each case, whereas, unexpectedly, neither antiserum lost its bactericidal activity (within the limits of experimental error) when absorbed with the GBMP. To avoid any ambiguities

in this data as a result of the possibility of substantial quantities of GBMP-specific antibodies remaining in the antisera after absorption with the GBMP, the absorbed antisera were subjected to RIA (6) using a  $^3\text{H}$ -labeled GBMP. No significant binding of the labeled GBMP to the absorbed antisera was detected. Clearly, the antibodies that were bactericidal for group B meningococcal organisms did not bind to the GBMP.

This result has extremely important implications in that there has been much discussion about the advisability of inducing GBMP-specific IgG antibodies in humans even if an immunogen to accomplish this was available. The objection is based primarily on the hypothesis that the poor immunogenicity of the GBMP is a natural self-protective phenomenon and is based on an epitope (a decasaccharide of  $\alpha$ -(2 $\rightarrow$ 8)-linked sialic acid) common to the GBMP (6) and human and animal tissue components (7, 8). This structural homology has been unambiguously demonstrated in the case of glycopeptides isolated from human and rat fetal brain tissue (7, 8), and the ability of these components to bind in vitro to GBMP-specific antibodies has been established (14, 15). Whether this objection is valid remains conjectural, and until there is conclusive evidence that this approach is detrimental, attempts to make an immunogenic form of the GBMP should not be abandoned. In the meantime we have demonstrated that although the *N*-Pr-GBMP-TT conjugate does raise crossreactive GBMP-specific antibodies in mice it also induces in them *N*-Pr-GBMP-specific antibodies that do not bind to the GBMP but that are specifically bactericidal for group B *N. meningitidis*.

In conclusion, it can be inferred from the above evidence that the *N*-Pr-GBMP mimics a unique bactericidal epitope on the surface of group B meningococcal organisms that is not present on exogenous GBMP. However, given the structural similarity between the *N*-Pr-GBMP and the GBMP it is likely that the GBMP, probably in a modified form on the bacterial surface, is still involved in the formation of the bactericidal epitope. Work is now in progress to define this potentially important epitope.

### Summary

Antibodies induced in mice by the *N*-propionyl (*N*-Pr)-group B meningococcal polysaccharide (GBMP)-tetanus toxoid (TT) conjugate were bactericidal for GBM organisms independent of protein serotype. The antisera contained two populations of *N*-Pr-GBMP-specific antibodies, only one of which crossreacted with the GBMP. Particularly significant was the fact that the bactericidal activity was mainly associated with the population of antibodies that did not crossreact with the GBMP. Therefore it can be inferred from the above evidence that the *N*-Pr-GBMP mimics a unique epitope on the surface of GBM organisms that is not present on the exogenous GBMP.

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