Specificity of Antibodies to O-Acetyl-Positive and O-Acetyl-Negative Group C Meningococcal Polysaccharides in Sera from Vaccinees and Carriers

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Most group C Neisseria meningitidis strains produce an O-acetyl-positive polysaccharide, a homopolymer of α-2→9-linked N-acetylneuraminic acid with O-acetyl groups at the C-7 and C-8 of its sialic acid residues. The majority of disease isolates have been reported to contain this polysaccharide. Some strains produce group C polysaccharide lacking O-acetyl groups. The licensed vaccine contains the O-acetyl-positive polysaccharide. We have measured the antibody specificities to the two polysaccharides in sera from asymptomatic group C meningococcal carriers and vaccinated adults by a new enzyme-linked immunosorbent assay (ELISA) procedure using methylated human serum albumin for coating the group C polysaccharide onto microtiter plates. Inhibition of binding of serum antibodies to polysaccharide-coated plates was measured by ELISA after incubation with O-acetyl-positive and O-acetyl-negative group C polysaccharides. Greater inhibition of binding of carrier sera was observed with the homologous polysaccharide. There was substantial inhibition of binding of vaccinee sera to the O-acetyl-positive polysaccharide-coated plate following preincubation with O-acetyl-positive polysaccharide, but homologous inhibition on plates coated with the O-acetyl-negative polysaccharide required much higher concentrations of polysaccharide. Carrier sera may have a higher proportion of antibodies with greater specificity for the O-acetyl-negative polysaccharide, while vaccinee sera contain antibodies with greater affinity for the O-acetyl-positive polysaccharide. Studies with monoclonal antibodies specific for O-acetyl-positive and O-acetyl-negative polysaccharides reveal that the percentage of group C meningococcal disease caused by O-acetyl-negative strains remains about 15%, as found over 15 years ago.

Group C Neisseria meningitidis is responsible for approximately 35% of meningococcal disease in the United States. N. meningitidis is carried asymptomatically in the upper respiratory tract by about 10% of the human population. Nasopharyngeal carriage of meningococcal strains as well as convalescence from the disease have been shown to result in the production of bactericidal antibodies associated with protective immunity (8, 13, 21, 28). The human nasopharynx is the only known reservoir of the organism, and most people who contract the disease do not do so by contact with an individual with meningococcal disease. Therefore, asymptomatic carriers are presumed to be the major source of transmission of pathogenic strains (6).

Many of the studies of meningococcal carriage have involved group B, for which it has been shown that acquisition and carriage of poorly pathogenic strains possessing serotype 2 surface proteins results in development of antibodies that could protect against a virulent group B serotype 2 strain (15). Although carriage of group B serotypes may confer some protection against group C meningococcal infections, little information is available on antibody responses and protective immunity due to carriage of group C N. meningitidis. Vaccinated individuals receive purified polysaccharide, whereas carriers and patients are exposed presumably to the same polysaccharide when it is an integral part of the bacteria. This provides us with an opportunity to study possible similarities and differences in the immune responses between these two groups.

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MATERIALS AND METHODS

**Bacteria and sera.** *N. meningitidis* group C strains C11, MC19, BB639, BB649, BB1098, BB1122, BB1146, BB620, BB657, BB666, BB688, BB1088, and BB1148 and strain M986-NCV-1 (a noncapsulated variant of *N. meningitidis* group B strain M986) were from the investigators' collection.

Sera from 119 adults, of which 6 were meningococcal group C carriers, 12 were group B carriers, and the rest were noncarriers, were provided to us for analysis by James Thomas of the Los Angeles County Public Health Department (27). Pre- and postvaccination sera were obtained from laboratory personnel. Acute- and convalescent-phase sera from children with group C meningococcal disease were from our laboratory collection. The two mouse monoclonal antibodies (2055.5 and 2016.3) used in this study were from Kathryn Stein and Leonard Rubinstein, Center for Biologics Evaluation and Research, Food and Drug Administration (22, 23).

**Purification of GCPS and outer membranes.** OAc+ and OAc− GCPS were prepared by the procedure of Gotschlich after strains C11 and MC19 were grown in modified Frantz medium (14). Briefly, bacterial cultures grown overnight were centrifuged, and 10% hexadecyl trimethyl ammonium bromide (Sigma Chemical Co., St. Louis, Mo.) was added to the supernatant to a final concentration of 0.1% to precipitate the polysaccharide. The pellet was suspended in 1 M chloride to dissolve the polysaccharide-detergent complex. A series of phenol extractions, ethanol precipitations, and high-speed centrifugations were done to purify the polysaccharide. OAc+ and OAc− GCPS were run on Sepharose CL-4B, and molecular sizes and elution profiles were similar for both. Purity of the polysaccharides was ascertained by sialic acid (26) and protein (19) analyses and also by the presence or absence of O-acetyl groups as determined by nuclear magnetic resonance spectroscopy, performed by William Egan, Center for Biologics Evaluation and Research, Food and Drug Administration. Outer membrane vesicles for use in ELISA were prepared from 65-h culture supernatants from strain M986-NCV-1 as described previously (9).

**Polysaccharide ELISA.** mHSA was prepared by the method of Mandell and Hershey (20) with 25% albumin (for injection) as the starting material. A mixture of mHSA and GCPS was made for coating Immulon 1 plates (Dynatech Laboratories, Inc., Chantilly, Va.) by diluting stock solutions of mHSA (5 mg/ml in water) and GCPS (1 mg/ml in water) in phosphate-buffered saline, pH 7.4 (PBS). After various concentrations were tried, a mixture of mHSA and GCPS each at a concentration of 5 μg/ml was found to be optimal, with minimum antibody binding to mHSA alone. The plates were coated for 6 to 7 h at 28°C and washed four times with saline containing 10 mM Tris (pH 7.5) and 0.1% Brij 35 (Sigma) by using an automatic plate washer (Dynatech). The standard reference serum (PB-2, assigned 2,400 U of GCPS antibody per ml) used in the assay was serially diluted in twofold steps, starting with an initial dilution of 1:100, in PBS containing 0.1% Brij 35 and 5% newborn calf serum (serum-conjugate buffer). Test sera were diluted appropriately in the same buffer to yield absorbance values within the linear region of the standard curve. Reference and test sera were applied to the plate in triplicate (100 μl per well) and incubated overnight at 4°C. The plates were washed the next day, and an appropriate dilution of goat anti-human immunoglobulin-alkaline phosphatase conjugate (affinity-purified immunoglobulin G [IgG] + IgM + IgA; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) in the serum-conjugate buffer was added to the plates (100 μl per well) and incubated at 28°C for 2 h. The plates were washed, and the color was developed with the addition of 100 μl (1 mg/ml) of p-nitrophenyl phosphate (Sigma no. 104) in 1 M Tris–0.3 mM MgCl2 (pH 9.8). The plates were read at 405 nm 50 to 60 min after the addition of the substrate. The recorded absorbance value was extrapolated to 100 min for normalization. Units of antibody in the test sera were determined by using a reference curve constructed by linear regression of logit-log-transformed data obtained with the PB-2 human reference serum (11). Serum PB-2 was obtained from an adult by plasmapheresis after immunization with meningococcal polysaccharide vaccine. A value of 2,400 U of anti-GCPS antibody per ml was assigned by comparison with a known anti-meningococcal outer membrane reference serum (10). Total antibodies to the outer membrane proteins (OMP) were measured by ELISA (11).

**Screening of group C meningococcal strains with monoclonal antibodies specific for OAc+ and OAc− GCPS.** Several disease isolates of group C *N. meningitidis* recovered from diverse geographic locations were grown, and their polysaccharide extracts were purified. Purity of the polysaccharide was estimated in each of these preparations by the resorcinol method (26). Equivalent amounts of sialic acid (5 μg/ml) from each strain were mixed with 5 μg of mHSA per ml, and 100 μl per well was coated on Immulon 1 plates. Half the plate was incubated with an appropriate dilution of monoclonal antibody specific for OAc+ GCPS, and the other half was incubated with monoclonal antibody specific for OAc− GCPS. After overnight incubation at 4°C, plates were washed and incubated with goat anti mouse IgG3-alkaline phosphatase conjugate (Southern Biotechnology Associates, Inc., Birmingham, Ala.) for 2 h at 28°C. Plates were processed as described above.

**Inhibition assays.** Inhibition studies were carried out by incubating twofold serial dilutions of OAc+ and OAc− GCPS starting from 10 μg/ml for 4 h at room temperature with a single serum dilution shown to be in the linear range of absorbance in a direct ELISA. The 4-h incubation of polysaccharide with the serum dilution was chosen after comparing incubations of 2 to 12 h. The binding appeared to reach an equilibrium by 4 h. The plates were slowly rotated on an orbital shaker during this time, after which 100 μl was transferred to an OAc+ GCPS-coated plate, and another 100 μl was transferred an OAc− polysaccharide-coated plate. The plates were incubated overnight at 4°C, washed, and then incubated with anti-human conjugate and processed as described above. Inhibition studies were also done with mouse monoclonal antibodies 2055.5 and 2016.3 in an identical manner, using goat anti-mouse IgG3 conjugate.

RESULTS

**GCPS ELISA.** Serial dilutions of sera from carriers and noncarriers were incubated with plates coated with a mixture of mHSA and GCPS to measure total antibodies to GCPS as described in Materials and Methods. Typical direct binding curves of absorbance plotted against serum dilution for sera from a carrier and a noncarrier are shown in Fig. 1. The differences in antibody levels between the two sera are evident from the figure.

**Measurement of antibodies to GCPS and OMP in carriers and noncarriers.** Sera from group C meningococcal carriers, group B carriers, and noncarriers were analyzed for antibodies against GCPS and OMP prepared from a nonencapsu-
luted variant of a group B strain (Table 1). Group C carriers had significantly higher antibodies to both GCPS and OMP than did non-group C carriers. In contrast, sera from group B carriers showed an increase in antibodies only against OMP. Although the overall values for noncarriers were low for antibodies against GCPS and OMP when compared with values for carriers, the range in antibody units per milliliter was large. Figure 2 is a bar graph depicting the percentages of noncarriers, group C carriers, and group B carriers in three different categories based on their antibody values against GCPS and OMP. The three categories were (i) high GCPS antibody and high OMP antibody, (ii) low GCPS antibody and high OMP antibody and, (iii) low GCPS antibody and low OMP antibody. These categories contained 12, 34, and 64%, respectively, of the noncarriers. The majority (83%) of group C carriers were in the first category, and 83% of group B carriers were in second category.

Measurement of antibodies to OAc⁺ and OAc⁻ GCPS by ELISA. Sera from noncarriers, group C carriers, and adults vaccinated with meningococcal tetravalent vaccine (OAc⁺ GCPS), as well as acute- and convalescent-phase sera from patients with group C meningococcal disease, were examined by ELISA using GCPS containing or lacking O-acetyl groups (Table 2). Although the binding of carrier and vaccinee sera to OAc⁻ GCPS-coated plates was slightly higher than binding to OAc⁺ GCPS-coated plates, the difference was not statistically significant.

Competitive inhibition assays with sera from carriers and vaccinees. Competitive inhibition ELISAs were done with sera from group C meningococcal carriers to measure the antibody responses specific for OAc⁺ and OAc⁻ GCPS (Fig. 3). Absorption with OAc⁺ polysaccharide inhibited the binding of carrier sera to the OAc⁺ polysaccharide-coated plate more than absorption with OAc⁻ polysaccharide, and comparably greater homologous inhibition was observed with the OAc⁻ polysaccharide-coated plates. Although vaccinee sera bound similarly to OAc⁺ and OAc⁻ polysaccharide-coated plates in the direct ELISA (Table 2), the competitive ELISA results were not as we had expected. Figure 4 shows the inhibition curves for two representative serum samples. The binding of serum A to OAc⁺ polysaccharide-coated plates was inhibited 80% with 10 μg of OAc⁺ polysaccharide per ml but only 15% with 10 μg of OAc⁻ GCPS per ml. At the same concentration, the OAc⁺ and OAc⁻ GCPS inhibited the binding to the OAc⁻ GCPS-coated plate by 80 and 70%, respectively. The inhibition of binding of serum B was similar, although the amount of antibody in serum B against the OAc⁻ GCPS was twice that against the OAc⁺ GCPS in a direct ELISA; serum A had equal binding to both polysaccharides. Thus, Fig. 4 illustrates that OAc⁺ GCPS was more efficient than OAc⁻ GCPS in inhibiting the binding of vaccinee sera to OAc⁻ GCPS-coated plates, which was not the case with carrier sera. The concentrations of OAc⁺ and OAc⁻ GCPS needed for 50% inhibition of binding of six carrier and four vaccinee sera to OAc⁺ and OAc⁻ GCPS were calculated from several inhibition curves (Table 4). The amounts of OAc⁺ GCPS required for 50% inhibition of binding of carrier and vaccinee sera to the OAc⁺ GCPS-coated plate were very similar, but there were significant differences in the amount required for inhibition of binding to the OAc⁻ GCPS-coated plate. The

### Table 1. Comparative antibody levels in sera of adults to group C meningococcal antigens as measured by ELISA

<table>
<thead>
<tr>
<th>Study group</th>
<th>n</th>
<th>Geometric mean antibody level, U/ml (1 SD confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GCPS (OAc⁺)</td>
</tr>
<tr>
<td>Noncarrier</td>
<td>97</td>
<td>2.1 (0.7–3.3); NS²</td>
</tr>
<tr>
<td>Group B carrier</td>
<td>12</td>
<td>1.2 (0.7–3.9); NS²</td>
</tr>
<tr>
<td>Group C carrier</td>
<td>6</td>
<td>19.0 (9.7–37.3); P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OMP</td>
</tr>
<tr>
<td>Noncarrier</td>
<td>97</td>
<td>7.7 (1.6–36.6); P &lt; 0.001</td>
</tr>
<tr>
<td>Group B carrier</td>
<td>12</td>
<td>40.2 (14.7–110.3); P &lt; 0.001</td>
</tr>
<tr>
<td>Group C carrier</td>
<td>6</td>
<td>45.7 (15.7–133.3); P &lt; 0.001</td>
</tr>
</tbody>
</table>

² Significance determined by Student's t test in comparison with noncarrier geometric mean antibody level. NS, not significant.
TABLE 2. Levels of antibodies to O-acetyl-positive and O-acetyl-negative GCPS in carriers, vaccinated adults, and patients

<table>
<thead>
<tr>
<th>Study group</th>
<th>n</th>
<th>Geometric mean antibody level, U/ml (1 SD confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OAc⁺</td>
</tr>
<tr>
<td>Noncarriers</td>
<td>4</td>
<td>1.85 (0.65–2.05)</td>
</tr>
<tr>
<td>Group C carriers</td>
<td>6</td>
<td>15.98 (9.22–37.13)</td>
</tr>
<tr>
<td>Vaccinated adults</td>
<td>11</td>
<td>30.69 (6.94–61.09)</td>
</tr>
<tr>
<td>Patients: acute</td>
<td>4</td>
<td>5.06 (0.50–7.68)</td>
</tr>
<tr>
<td>Patients: convalescent</td>
<td>5</td>
<td>12.23 (2.63–13.47)</td>
</tr>
</tbody>
</table>

The concentration of OAc⁻ GCPS needed for 50% inhibition of binding of vaccinee sera to the OAc⁻ GCPS-coated plate was significantly higher than that needed for inhibition of binding of carrier sera.

Monoclonal antibodies with specificity for OAc⁺ and OAc⁻ GCPS. Figure 5 illustrates the specificity of binding of monoclonal antibody 2055.5 to OAc⁺ GCPS. In a direct ELISA, this monoclonal antibody did not bind to the OAc⁻ GCPS-coated plate. Incubation of 2055.5 with OAc⁻ GCPS did not result in inhibition of binding to the OAc⁺ GCPS-coated plate either after incubation overnight at 4°C or for 4 h at room temperature. Another monoclonal antibody,
FIG. 4. Inhibition of binding of vaccinee sera A and B to OAc+ and OAc− GCPS-coated Immulon 1 plates. Vaccinee sera were preincubated with OAc+ and OAc− GCPS for 4 h and then transferred to OAc+ and OAc− GCPS-coated plates. Inhibitor: O and □, OAc+ GCPS; Δ and +, OAc− GCPS.

2016.3, did not bind to the OAc+ GCPS-coated plate (data not shown) and exhibited a very similar inhibition pattern with OAc− GCPS, and OAc+ polysaccharide did not inhibit the binding of the monoclonal antibody to the OAc− GCPS coated plate (data not shown).

Screening of meningococcal strains with monoclonal antibodies 2055.5 and 2016.3. Thirteen recent isolates of group C N. meningitidis from various geographic regions of the United States were examined for the presence of O-acetyl determinants (Table 3). Eleven of the 13 reacted only with monoclonal antibody 2055.5, and two reacted with both monoclonal antibodies 2016.3 and 2055.5. Polysaccharides isolated from these two strains, BB 1088 and BB 1146, were found to be O-acetyl negative by 13C nuclear magnetic resonance analysis. Whole-cell ELISA was also performed with each of these strains, but the data were not conclusive, as some of the strains made much less polysaccharide than others; therefore, additional studies were done after the polysaccharides were purified from each strain.

DISCUSSION

Several different methods for attaching polysaccharides to the solid phase for ELISA have been attempted, such as
tyramination of the polysaccharide and covalent attachment of a positively charged molecule like poly-L-lysine (4). These methods have the disadvantage that they can alter the native conformation of the polysaccharide. Precoating the plate with poly-L-lysine seemed ideal, but we found poly-L-lysine to react nonspecifically with some human sera and therefore to be unsuitable. Use of mHSA in attaching the polysaccharide to Immulon 1 plates was found to alleviate much of the nonspecific binding found with poly-L-lysine (17).

Carriage of group C meningococci increased the levels of antibodies to both GCPS and OMP (Table 1). The extent of the increase is probably dependent on the duration of carriage and the amount of polysaccharide made by the carrier strains. The antibody response may also be dependent on earlier exposure to other bacteria sharing antigenic determinants with the GCPS. Known meningococcal carriers uniformly had high levels of antibody to OMP. About 12% of the noncarriers had high levels of antibodies to both the GCPS and OMP and therefore may have been recent group C meningococcal carriers (Fig. 2). Of the noncarriers, 34% had a high level of antibody to the OMP but low levels to GCPS and presumably were recent carriers of meningococci other than group C. In contrast, a few had high levels of antibody to the GCPS and low levels to OMP. These individuals presumably had recent exposure to another bacteria with a structurally related polysaccharide. However, vaccination induced higher levels of antibody to the GCPS than either meningococcal carriage or exposure to other cross-reactive polysaccharides.

It had been observed in earlier studies that the OAc\(^+-\) GCPS may be more immunogenic in children and adults than the OAc\(^+\) polysaccharide. Individuals vaccinated with a preparation made from OAc\(^-\) GCPS had twice the amount of anticapsular as well as bactericidal antibodies as did individuals vaccinated with OAc\(^+\) polysaccharide, although the difference was not statistically significant (12, 25). In these studies, the antibodies were measured in the sera generated from vaccination with OAc\(^+\) and OAc\(^-\) GCPS by radioimmunoassay with only OAc\(^+\) GCPS. In our study, levels of binding of humoral antibodies from group C carriers and from vaccinated individuals to OAc\(^+\) and OAc\(^-\) GCPS were similar in a direct ELISA (Table 2).

Inhibition curves for carrier sera (Fig. 3) showed better inhibition with the respective homologous polysaccharides, as anticipated, and indicated that the heterogeneity in the inhibition patterns of OAc\(^+\) and OAc\(^-\) GCPS was due to at least in part to differences in specificity. Homologous inhibition of binding of antibodies from vaccinees to the OAc\(^-\)

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**TABLE 3.** Screening of \textit{N. meningitidis} strains with monoclonal antibodies specific for OAc\(^+\) and OAc\(^-\) GCPS

<table>
<thead>
<tr>
<th>Strain</th>
<th>Yr isolated</th>
<th>State</th>
<th>Sero group</th>
<th>Sero type</th>
<th>Binding with monoclonal antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2055.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(OAc(^+))</td>
</tr>
<tr>
<td>BB617</td>
<td>1987</td>
<td>Okla.</td>
<td>C</td>
<td>2aP1.2</td>
<td>+</td>
</tr>
<tr>
<td>BB620</td>
<td>1987</td>
<td>Wash.</td>
<td>C</td>
<td>2aP1.2</td>
<td>+</td>
</tr>
<tr>
<td>BB639</td>
<td>1987</td>
<td>Calif.</td>
<td>C</td>
<td>2aP1.2</td>
<td>+</td>
</tr>
<tr>
<td>BB649</td>
<td>1987</td>
<td>N.J.</td>
<td>C</td>
<td>2aP1.2</td>
<td>+</td>
</tr>
<tr>
<td>BB657</td>
<td>1987</td>
<td>Calif.</td>
<td>C</td>
<td>NTP1.2</td>
<td>+</td>
</tr>
<tr>
<td>BB666</td>
<td>1987</td>
<td>Tenn.</td>
<td>C</td>
<td>NTP1.2</td>
<td>+</td>
</tr>
<tr>
<td>BB688</td>
<td>1987</td>
<td>Calif.</td>
<td>C</td>
<td>2aP1.2</td>
<td>+</td>
</tr>
<tr>
<td>BB1088</td>
<td>1986</td>
<td>Mich.</td>
<td>C</td>
<td>NT nt</td>
<td>+</td>
</tr>
<tr>
<td>BB1098</td>
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<td>N.J.</td>
<td>C</td>
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</tr>
<tr>
<td>BB1122</td>
<td>1987</td>
<td>Ohio</td>
<td>C</td>
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<td>+</td>
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<tr>
<td>BB1146</td>
<td>1984</td>
<td>Nev.</td>
<td>C</td>
<td>2a nt</td>
<td>+</td>
</tr>
<tr>
<td>BB1147</td>
<td>1984</td>
<td>N.Y.</td>
<td>C</td>
<td>NTP1.2</td>
<td>+</td>
</tr>
<tr>
<td>BB1148</td>
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<td>C</td>
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<td>+</td>
</tr>
<tr>
<td>C11</td>
<td></td>
<td></td>
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<td>+</td>
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<td>MC19</td>
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<td>C</td>
<td></td>
<td>+</td>
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<td>M986</td>
<td>B</td>
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<td>2aP1.2</td>
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<td>-</td>
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TABLE 4. Concentrations of GCPS producing 50% inhibition of antibody binding to OAc+ and OAc− GCPS

<table>
<thead>
<tr>
<th>Serum</th>
<th>OAc+ GCPS bound</th>
<th>OAc− GCPS bound</th>
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<tbody>
<tr>
<td></td>
<td>Conc (µg/ml)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>yielding 50% inhibition by:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OAc+ GCPS</td>
<td>OAc− GCPS</td>
</tr>
<tr>
<td>Carrier sera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.1</td>
<td>&gt;10.0&lt;sup&gt;a&lt;/sup&gt; /</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>0.6</td>
<td>&gt;10.0</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>&gt;10.0</td>
</tr>
<tr>
<td>5</td>
<td>0.8</td>
<td>2.3</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>1.5</td>
<td>5.0</td>
</tr>
<tr>
<td>1 SD confidence interval</td>
<td>(0.6–3.8)</td>
<td>(2.1–11.7)</td>
</tr>
<tr>
<td>Vaccinee sera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.0</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>3.5</td>
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<td>3</td>
<td>2.0</td>
<td>&gt;10.0</td>
</tr>
<tr>
<td>4</td>
<td>0.7</td>
<td>&gt;10.0</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>1.7</td>
<td>10.0</td>
</tr>
<tr>
<td>1 SD confidence interval</td>
<td>0.9–3.4</td>
<td>10.0–10.0</td>
</tr>
<tr>
<td>Significance&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Values of >10 were considered as 10 for calculation of geometric means.

<sup>b</sup> Significance determined by Student’s t test by comparing geometric mean concentrations (micrograms per milliliter) for carrier and vaccine sera when inhibited by (i) OAc+ GCPS and the bound GCPS is OAc+ (ii) OAc− GCPS and the bound GCPS is OAc−, (iii) OAc+ GCPS and the bound GCPS is OAc−, and (iv) OAc− GCPS and the bound GCPS is OAc+.

A small study in the 1970s suggested that most disease isolates contained the OAc− GCPS. Our limited data obtained by using monoclonal antibodies specific for OAc+ and OAc− GCPS suggest that approximately 85% of recent group C meningococcal disease isolates from the United States express OAc+ polysaccharide (Table 3). More strains have to be screened to confirm this preliminary observation. However, there are no data available on the proportion of carrier strains that are OAc+ or OAc−. It would be interesting to see if the proportion of OAc− and OAc− specific antibodies in a carrier serum is dependent on whether the carrier strain produced polysaccharide with or without O-acetyl groups and if there are more carrier strains than disease strains lacking O-acetyl groups.

The inhibition studies with sera from carriers and vaccinated adults and monoclonal antibodies show that the OAc+ and OAc− polysaccharides share at least one common backbone epitope. The absence and presence of O-acetyl groups generate at least one unique epitope on each of the two polysaccharides (22, 23). The proportions of epitopes common and unique to OAc+ and OAc− GCPS in carrier and vaccinee sera are probably different, as vaccinees receive only OAc+ GCPS. It is also possible that the OAc− GCPS has a more stable conformation on a solid surface than in solution, as indicated by a decrease in the amount of OAc− GCPS needed for 50% inhibition of binding of vaccinee sera when incubated with OAc+ polysaccharide coated on a microtiter plate (data not shown). In this regard, we found that the fine specificities of some monoclonal antibodies changed depending upon whether the GCPS was bound directly to the plastic (Immuno 2) or through interaction with a protein (mHSA; unpublished results). This may be the same effect we observed when soluble OAc− GCPS failed to effectively inhibit binding of human antibodies to the bound mHSA-GCPS complex.

The present studies thus demonstrate that exposure to the GCPS induces antibodies of multiple specificities. The nature of this exposure, i.e., carriage, disease, or vaccination, has a strong impact upon the predominant antibody specificity.

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REFERENCES


