Naturally Acquired Passive Protective Activity against *Neisseria meningitidis* Group C in the Absence of Serum Bactericidal Activity

Jo Anne Welsch and Dan Granoff*

*Children’s Hospital Oakland Research Institute, Oakland, California*

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The hallmark of immunity to meningococcal disease is a bactericidal titer in serum of $\geq$1:4 measured with human complement, but this threshold titer may underestimate the extent of protection. We used the infant rat model of meningococcal bacteremia to measure group C passive protective activity in serum samples from 91 unimmunized adults living in California. A total of 35 sera (38.5%) had passive protective activity. Sera with complement-mediated bactericidal titers of $\geq$1:4 were 3.4-fold more likely to confer protection (89%) than nonbactericidal sera (26%; $P < 0.0001$). Thus, bactericidal titers of $\geq$1:4 are a marker of protection, but this threshold lacks sensitivity for predicting protective activity. We investigated the 73 sera with bactericidal titers of $<1:4$ to determine the basis of protective activity. The 19 sera with protective activity had a higher geometric mean group C anticapsular antibody concentration ($0.72 \mu g/ml$) than the 54 sera that lacked protective activity ($0.16 \mu g/ml; P < 0.001$). Thus, protective activity in the absence of bactericidal activity was associated with higher concentrations of anticapsular antibodies, but not all sera with anticapsular antibodies conferred protection. Of 18 nonbactericidal sera with anticapsular antibody concentrations between 0.31 and 0.99 $\mu g/ml$, the 11 sera that conferred protection had a higher mean antibody avidity constant ($21.9 \text{nM}^{-1}$) than the 7 nonprotective sera ($14.6 \text{nM}^{-1}; P < 0.03$). Thus, in sera with titers of $<1:4$, protective activity is associated with higher-avidity group C anticapsular antibodies, which are present in concentrations insufficient to elicit complement-mediated bacteriolysis in vitro but sufficient to confer protection in an in vivo bacteremia model.

New multivalent meningococcal polysaccharide-protein conjugate vaccines are currently in development (5, 30) and will likely be licensed in Europe and North America in the next few years (28). The low incidence of meningococcal disease in these populations precludes performing prospective randomized clinical trials to determine the efficacy of these new vaccines. Vaccine efficacy, therefore, will be inferred from immunogenicity data (3), and vaccine effectiveness will be confirmed in subsequent postlicensure studies (1), following a licensure pathway and monitoring strategies adapted in the United Kingdom for the introduction of group C meningococcal conjugate vaccines. There is a strong scientific basis for inferring meningococcal vaccine efficacy from immunogenicity data (3, 10, 11). However, the choice of in vitro assay conditions and serologic endpoints for inferring protection against meningococcal disease are topics of considerable recent debate (1, 3, 16, 32). The reasons are complex but ultimately have to do with the effects of potential disparities between in vitro antibody functional assay conditions and in vivo host defenses.

Meningococci grown in vivo likely express different genes than those of bacteria grown in vitro (13). Also, when meningococci are grown in broth or agar, the choice of growth conditions may affect capsular production and/or the expression of different surface proteins or lipoooligosaccharide structures (6, 22, 23, 35), which in turn can affect the susceptibility of the bacterial cell to antibody binding and complement-mediated bacteriolysis. These factors may limit the interpretation of the results of in vitro antibody functional studies. Members of our laboratory recently described an infant rat meningococcal bacteremia model for measuring antibody protective activity against group B or C strains (15, 25). Although meningococci are obligate human pathogens with species-specific pathogenic mechanisms (17), the infant rat model permits the investigation of the protective activity of antibodies in a setting where the organism is rapidly replicating in vivo.

In the present study, we used the infant rat model to investigate the role of naturally acquired serum antibodies of human adults in protection against group C meningococcal disease. Protective activity in serum measured in vivo was related to the presence or absence of group C complement-mediated bactericidal activity measured in vitro or to the concentrations and avidities of group C anticapsular antibodies in serum. The results provide insights into the antigenic targets of naturally acquired antibodies conferring protection against group C *Neisseria meningitidis* and the extent to which measurements of serum bactericidal activity may underestimate protective immunity.

**MATERIALS AND METHODS**

**Serum samples.** We used a convenience sample of 91 stored preimmunization sera that had been obtained from healthy adults ranging in age from 18 to 58 years who were enrolled in meningococcal vaccine immunogenicity trials conducted at Children’s Hospital and Research Center at Oakland between 2001 and 2003. None of the subjects had been previously immunized with meningococcal vaccine. To preserve internal complement activity, the blood was allowed to clot at room temperature for 30 min and centrifuged at 2,135 $\times g$ for 10 min at 4°C. The sera were promptly separated, divided into 0.5-ml aliquots, and stored frozen at $-70^\circ$C. Use of these sera for the present study was approved by

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* Corresponding author. Mailing address: 5700 Martin Luther King Jr. Way, Oakland, CA 94609. Phone: (510) 450-7640. Fax: (510) 450-7915. E-mail: dgranoff@chori.org.
the Institutional Review Board of Children’s Hospital and Research Center at Oakland.

Serology. (i) Bactericidal assay. The test strain was N. meningitidis strain 4243 (C:2a:P1.5,2), a member of the electrophoretic type 37 complex, sequence type 11 (http://www.mlst.net), expressing a polysaccharide capsule that is O acetylation positive (15). The organism was grown in Mueller-Hinton broth (with a starting $A_{600}$ of ~0.1) for approximately 2 h to an $A_{600}$ of ~0.6. After the bacteria were washed twice in Dulbecco’s buffer (pH 7.40) containing bovine serum albumin (Sigma, St. Louis, Mo.), approximately 300 to 400 CFU was added to the reaction mixture. In the exogenous complement assay, the final 60 µl reaction mixture contained 20% (vol/vol) complement and serial twofold dilutions of test sera diluted in Dulbecco’s buffer. The human complement was serum from a healthy adult with no detectable endogenous group C bactericidal activity and no detectable group C anticapsular antibody as measured by a radioligand binding assay (RABA; see below). The rabbit complement consisted of pooled infant rabbit serum that lacked endogenous bactericidal activity (Cedarlane; Hornby, Ontario, Canada). The percent survival of bacteria after 60 min of incubation in the reaction mixture compared to the number of CFU per milliliter in the negative control serum at time zero was plotted against serum dilution. The bactericidal titer was defined by the 50% intercept. The bactericidal assay using internal complement was performed as described above except that we used single dilutions of test sera (final dilution of 1:4) and did not add exogenous complement.

(ii) RABA. The concentrations of group C anticapsular antibodies were measured by a RABA performed as previously described (15). For measurement of antibody avidity, we performed replicate assays using two different concentrations of radiolabeled antigen (15). Avidity constants ($K_a$) were calculated by comparing the fraction of antigen bound at different antibody concentrations in the low-antigen-concentration RABA to that of the total concentration of antibody determined in the high-antigen-concentration RABA (15).

(iii) Passive protection. The animal model has been described previously (15, 26). In brief, 5- to 7-day-old pups from litters of outbred Wistar rats (Charles River, Raleigh, N.C.) were randomly redistributed to the nursing mothers. At time zero, 100 µl of a 1:4 dilution of serum was administered intraperitoneally (i.p.) (3 rats/serum sample). Two hours later, the animals were challenged i.p. with 100 µl of washed, log-phase N. meningitidis group C strain 4243 cells (range in different experiments, ~800 to 1,400 CFU/rat). Eighteen hours after the bacterial challenge, blood specimens were obtained by puncturing the heart with a syringe and needle containing approximately 25 µl of heparin without preservative (American Pharmaceutical Partners, Inc., Los Angeles, Calif.). Aliquots of 1, 10, and 100 µl of blood were plated onto chocolate agar. The number of CFU per milliliter of blood was determined after overnight incubation of the plates at 37°C in 5% CO₂. Protection was defined as a 2-log decrease in the geometric mean number of CFU/ml of blood in the triad of animals given the test serum compared to that of a group of negative control animals (geometric means, ~100 to 544,000 CFU/ml in different experiments).

Statistical analysis. The geometric means of the antibody concentrations or titers were computed by exponentiation (base 10). For log transformation, samples below the lower limits of detection were assigned a value of half of the lower limit (i.e., 1.2 for the bactericidal titer and 0.05 µg/ml for group C anticapsular antibody concentration measured by RABA). The proportion of sera with bactericidal titers of ≥1:4 (considered a protective titer [10]) or ≥1:128 when measured with rabbit complement (a titer previously shown to predict a bactericidal titer of ≥1:4 when measured with human complement [32]) was computed. Differences found in the proportion of subjects in the respective groups were compared by chi-square analysis. For determination of sensitivity and specificity, sera with passive protective activity in the rat model were considered “true positives” of immunity to group C meningococcal disease. Sensitivity (percent) was defined by the equation (number of sera with positive serology/number of protective sera) × 100. Specificity (percent) was defined by the equation (number of sera with negative serology/number of sera that lacked protective activity) × 100.

RESULTS

Passive protective activity in infant rats. Fig. 1 shows the results of a representative experiment measuring the kinetics and time course of bacteremia in 5-day-old infant rats challenged i.p. with group C strain 4243. The level of bacteremia increased exponentially for ~8 h, reaching >250,000 CFU/ml. As shown in Table 1, pretreatment of the rats with human serum 2 h before bacterial challenge can confer protection against bacteremia. In the examples shown, a 1:26 dilution of a positive control serum from an adult immunized with meningococcal polysaccharide vaccine conferred protection, as did 1:4 dilutions of preimmunization sera from subjects 1 and 3 but not subjects 2 and 4. Of the 91 preimmunization sera tested, 35 sera (38%) conferred passive protection in the infant bacteremia model and 56 sera (62%) showed no significant protective activity (<2-log decrease in the geometric mean number of CFU/ml compared to that of the negative control animals). As summarized in Table 2, the demographics of the groups whose sera did and did not confer protection were similar. The geometric means of the bactericidal titers and group C anticapsular antibody concentrations were significantly higher in the protective sera than in the nonprotective sera ($P < 0.0001$).

Relationship between serum complement-mediated bactericidal activity and passive protective activity in the infant rat model. Of the 91 sera, 73 sera (80.2%) had titers of <1:4, measured with exogenous human complement, and 18 sera (19.8%) had bactericidal titers of ≥1:4 (Table 3). We performed absorption studies on 16 of the bactericidal sera using 25 µg of soluble group C polysaccharide/ml, which was sufficient to give 100% inhibition of high-titer bactericidal control sera from an adult immunized with meningococcal polysaccharide vaccine. Seven of the bactericidal sera (44%) were completely inhibited by the polysaccharide, and nine sera (66%) were not inhibited. Five of the sera that were not inhibited had low anticapsular antibody concentrations measured by RABA (<0.33 µg/ml), a result consistent with the bactericidal antibodies being directed at noncapsular antigens. The remaining four sera that were not inhibited had anticapsular antibody concentrations of >1 µg/ml, and it is likely that both anticap-
and anticapsular antibody concentrations were higher in the protective sera than in the nonprotective sera \( (P = 0.0001) \). Quantitative blood cultures were obtained at 18 h. The negative and positive control sera were obtained before and 1 month after meningococcal polysaccharide vaccination of an adult and were tested for protective activity at a dilution of 1:4 and 1:26, respectively. All other sera were tested at a dilution of 1:4. Protection was defined by a >2-log decrease in the geometric mean number of CFU per milliliter of three replicate animals given the test serum compared to that of the group of animals treated with the negative control serum.

Sera with bactericidal titers of ≥1:4 were 3.4-fold more likely to confer passive protective activity against bacteremia in the rat model (16 of 18 sera [88.9%]) than nonbactericidal sera (19 of 73 sera [26%]; \( P < 0.0001 \) by the chi-square test). As summarized in Table 3, the proportion of sera with bactericidal titers between 1:4 and 1:6 that conferred protection (5 of 6) was not significantly different from that of sera with bactericidal titers of ≥1:20 (11 of 12). Note that there were no sera with titers between 1:7 and 1:19 when sera were assayed with exogenous human complement.

Serum bactericidal activity was also measured with internal human complement, which was used in the Goldschneider et al. study (10), or exogenous rabbit complement, which because of ease of standardization is currently used by most laboratories to measure bactericidal titers (1, 3) but is known to give higher bactericidal titers than human complement (3, 32). Irrespective of the source of complement used, protective activity was more frequently found in sera that were positive for bactericidal activity (with a titer of ≥1:4 with human complement or ≥1:128 with rabbit complement) than in sera that were negative for bactericidal activity (\( P < 0.001 \)).

The specificities of sera with bactericidal titers of ≥1:4 when measured with internal or external human complement were 91 and 96%, respectively, for passive protective activity (Table 4). However, a titer positive for bactericidal activity was not very sensitive for passive protective activity (40 and 46% when measured with internal and external human complement, respectively), since 26 to 29% of sera with bactericidal titers of <1:4 conferred protection in the animal model (Table 3). When bactericidal titers were measured with exogenous infant rabbit complement instead of human complement, a titer of ≥1:128 was 60% sensitive and 93% specific for protective activity (Table 4).

### TABLE 1. Passive protective activity of representative adult sera in infant rats challenged i.p. with *N. meningitidis* group C strain 4243*<sup>a</sup>

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>Bactericidal titer&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Blood culture result at 18 h</th>
<th>Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>&lt;1:4</td>
<td>5/5</td>
<td>No</td>
</tr>
<tr>
<td>Positive control</td>
<td>1:15</td>
<td>0/5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Subject 1</td>
<td>&lt;1:4</td>
<td>0/3</td>
<td>Yes</td>
</tr>
<tr>
<td>Subject 2</td>
<td>&lt;1:4</td>
<td>3/3</td>
<td>No</td>
</tr>
<tr>
<td>Subject 3</td>
<td>1:6</td>
<td>2/3</td>
<td>Yes</td>
</tr>
<tr>
<td>Subject 4</td>
<td>1:6</td>
<td>3/3</td>
<td>No</td>
</tr>
</tbody>
</table>

<sup>a</sup> Five-day-old infant rats were given 0.1 ml of serum i.p. at time zero. Two hours later, animals were challenged i.p. with ~900 CFU of *N. meningitidis* group C strain 4243. Quantitative blood cultures were obtained at 18 h. The negative and positive control sera were obtained before and 1 month after meningococcal polysaccharide vaccination of an adult and were tested for protective activity at a dilution of 1:4 and 1:26, respectively. All other sera were tested at a dilution of 1:4. Protection was defined by a >2-log decrease in the geometric mean number of CFU per milliliter of three replicate animals given the test serum compared to that of the group of animals treated with the negative control serum.

<sup>b</sup> Tested with exogenous human complement.

Sera with bactericidal titers of ≥1:4 were 3.4-fold more likely to confer passive protective activity against bacteremia in the rat model (16 of 18 sera [88.9%]) than nonbactericidal sera (19 of 73 sera [26%]; \( P < 0.0001 \) by the chi-square test). As summarized in Table 3, the proportion of sera with bactericidal titers between 1:4 and 1:6 that conferred protection (5 of 6) was not significantly different from that of sera with bactericidal titers of ≥1:20 (11 of 12). Note that there were no sera with titers between 1:7 and 1:19 when sera were assayed with exogenous human complement.

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The specificities of sera with bactericidal titers of ≥1:4 when measured with internal or external human complement were 91 and 96%, respectively, for passive protective activity (Table 4). However, a titer positive for bactericidal activity was not very sensitive for passive protective activity (40 and 46% when measured with internal and external human complement, respectively), since 26 to 29% of sera with bactericidal titers of <1:4 conferred protection in the animal model (Table 3). When bactericidal titers were measured with exogenous infant rabbit complement instead of human complement, a titer of ≥1:128 was 60% sensitive and 93% specific for protective activity (Table 4).

**Relationship between serum anticapsular antibody concentrations and passive protective activity in infant rats.** Of the 91 sera, 50 sera had group C anticapsular antibody concentrations of ≥0.3 µg/ml as measured by RABA. 21 sera had anticapsular antibody concentrations between 0.31 and 0.99 µg/ml, and 20 sera had concentrations of ≥1.0 µg/ml. The respective proportions of sera with passive protective activity in the infant rat model were 12, 62, and 80%. Thus, the proportion of sera with passive protective activity increased with increasing group C anticapsular antibody concentration (\( P < 0.0001 \) by the chi-square test for trend). The specificity and sensitivity of a serum anticapsular antibody concentration of ≥1 µg/ml for passive protective activity were 93 and 46%, respectively, values nearly identical to the specificity and sensitivity of a bactericidal titer of ≥1:4 measured with human complement (Table 4). As expected, at lower concentrations of anticapsular antibodies in serum, there was increased sensitivity but lower specificity for passive protective activity (Table 4).

Of the 50 sera with anticapsular antibody concentrations of ≥0.3 µg/ml, 6 sera (12%) conferred passive protection in the animal model. Given these low anticapsular antibody concentrations, the protective antibodies in these sera were likely directed at noncapsular antigens. Of the 21 sera with anticapsular antibody concentrations between 0.31 and 0.99 µg/ml, 13 sera conferred passive protection against bacteremia in the infant rat model and 8 were not protective. The respective geometric mean anticapsular antibody concentrations of protective and nonprotective sera were not significantly different (0.59 and 0.52 µg/ml; \( P > 0.4 \)). One possible explanation for why some sera with anticapsular antibody levels in this range were protective while others were not is a difference in anti-

### TABLE 2. Demographic characteristics of the adults whose sera were used and antibody concentrations in serum in relation to passive protective activity

<table>
<thead>
<tr>
<th>Serum type (no. of subjects)</th>
<th>Range (mean of subject age, in yr)</th>
<th>% Female</th>
<th>% White</th>
<th>% Health-care workers</th>
<th>Serum bactericidal activity (geometric mean titer)</th>
<th>Anticapsular antibody, geometric mean concen (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protective (n = 35)</td>
<td>18–57 (33.1)</td>
<td>46</td>
<td>74</td>
<td>27</td>
<td>7.6</td>
<td>1.46</td>
</tr>
<tr>
<td>Not protective (n = 56)</td>
<td>18–58 (30.2)</td>
<td>50</td>
<td>73</td>
<td>32</td>
<td>2.1</td>
<td>0.16</td>
</tr>
</tbody>
</table>

<sup>a</sup> There were no significant differences between the demographic characteristics of the two groups (\( P > 0.7 \)). The respective geometric mean serum bactericidal titers and anticapsular antibody concentrations were higher in the protective sera than in the nonprotective sera (\( P < 0.0001 \)).
capsular avidity, which was significantly higher in the 13 protective sera \( K_a \) (mean ± standard error) of \( (22.1 \pm 1.8 \text{ nM})^{-1} \) than in the 8 nonprotective sera \( K_a \) of \( (15.5 \pm 1.9 \text{ nM})^{-1} ; P = 0.02 \) by \( t \) test. Although this difference in mean avidity may seem too small to explain the difference in protective activity between the two groups, the results are consistent with previous data on the magnitude of avidity differences between highly protective and poorly protective vaccine-induced group C anticapsular antibodies (12, 15).

Characterization of protective antibodies in sera with bactericidal titers of <1:4 measured with exogenous human complement. Of the 73 sera that lacked bactericidal activity when measured with exogenous human complement, 19 sera conferred passive protection against bacteremia in the infant rat model and 54 sera did not. The group C anticapsular antibody concentrations of the individual samples are shown graphically in Fig. 2A. The protective sera had, on average, 4.5-fold-higher group C anticapsular antibody concentrations than sera that lacked protective activity (geometric means of 0.72 and 0.16 \( \mu g/ml \), respectively; \( P < 0.0001 \)). The group C antibody avidity constants for protective and nonprotective sera with anticapsular antibody concentrations of >0.3 \( \mu g/ml \) are shown in Fig. 2B (avidity could not be determined in samples with lower concentrations). For samples with anticapsular antibody concentrations between 0.31 and 0.99 \( \mu g/ml \), the 11 protective sera with bactericidal titers of <1:4 had on average higher-avidity anticapsular antibodies than did the 7 nonprotective sera \( K_a \) (mean ± standard error) of \( (21.9 \pm 2.0 \text{ nM})^{-1} \) versus \( (14.6 \pm 2.0 \text{ nM})^{-1} ; P < 0.03 \). Although there were no significant differences between the mean avidities of the protective and nonprotective sera with anticapsular antibody concentrations of \( \geq 1.0 \mu g/ml \), there were only four nonprotective sera in this group. One of these four sera had low avidity, and two sera with higher avidities had relatively high immunoglobulin A (IgA) anticapsular antibody concentrations measured by enzyme-linked immunosorbent assay (0.83 and 0.55 \( \mu g/ml \)), which could explain their lack of protective activity. We do not have an explanation for the lack of protection of the remaining serum sample in this group. Nevertheless, taken together the data suggest that most of the protective activity in sera with bactericidal titers of <1:4 results from subbactericidal concentrations of higher-avidity anticapsular antibodies.

DISCUSSION

Studies of naturally acquired meningococcal antibodies in the population have provided important insights into the immunologic basis of meningococcal immunity (reviewed in reference 27). For example, adults have a much lower incidence of meningococcal disease than children less than 4 years of age (28), which is thought to be a result of an age-related acquisition of serum antibodies stimulated by asymptomatic colonization by \textit{N. meningitidis} strains (11, 29) or cross-reacting bacteria (7, 37).

### TABLE 3. Serum protective activity in relation to bactericidal activity assayed with different complement sources

<table>
<thead>
<tr>
<th>Complement</th>
<th>Bactericidal titer</th>
<th>No. of serum samples</th>
<th>Number (%) of sera that were:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protective</td>
</tr>
<tr>
<td>Exogenous human</td>
<td>&lt;1:4</td>
<td>73</td>
<td>19 (26)</td>
</tr>
<tr>
<td></td>
<td>1:4–1:6</td>
<td>6</td>
<td>5 (83)</td>
</tr>
<tr>
<td></td>
<td>1:7–1:19</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>≥1:20</td>
<td>12</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Internal human</td>
<td>&lt;1:4</td>
<td>72</td>
<td>21 (29)</td>
</tr>
<tr>
<td></td>
<td>≥1:4</td>
<td>19</td>
<td>14 (74)</td>
</tr>
<tr>
<td>Exogenous rabbit</td>
<td>&lt;1:16</td>
<td>64</td>
<td>13 (20)</td>
</tr>
<tr>
<td></td>
<td>1:16–1:127</td>
<td>2</td>
<td>1 (50)</td>
</tr>
<tr>
<td></td>
<td>≥1:1:28</td>
<td>25</td>
<td>21 (84)</td>
</tr>
</tbody>
</table>

* With each of the complement sources, the difference between the proportion of bactericidal sera with protective activity and the respective proportion of nonbactericidal sera with protective activity is significant (\( P < 0.0004 \) by the chi-square test). NA, not applicable because no sera had titers between 1:7 and 1:19.

### TABLE 4. Sensitivities and specificities of serum bactericidal activities or different concentrations of group C anticapsular antibodies for passive protective activity in the infant rat bacteremia model

<table>
<thead>
<tr>
<th>Bactericidal titer or antibody concn</th>
<th>Complement source</th>
<th>Passive protective activity percent (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Bactericidal titer in serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1:4</td>
<td>Human, internal</td>
<td>40 (30–50)</td>
</tr>
<tr>
<td>≥1:4</td>
<td>Human, exogenous</td>
<td>46 (36–56)</td>
</tr>
<tr>
<td>≥1:128</td>
<td>Rabbit, exogenous</td>
<td>60 (50–70)</td>
</tr>
<tr>
<td>Anticapsular antibody concn (( \mu g/ml ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥0.3</td>
<td>NA</td>
<td>86 (78–92)</td>
</tr>
<tr>
<td>≥0.5</td>
<td>NA</td>
<td>69 (59–78)</td>
</tr>
<tr>
<td>≥1.0</td>
<td>NA</td>
<td>46 (36–56)</td>
</tr>
</tbody>
</table>

* Anticapsular antibody concentrations were measured by RABA. NA, not applicable. The 95% CIs were calculated from binomial distributions.
In their seminal study, Goldschneider et al. demonstrated the importance of serum bactericidal antibodies in protection against group C meningococcal disease during an epidemic among military recruits in the 1960s (10). Group C bactericidal antibodies were present in baseline sera of approximately 82% of the recruits. The subjects with detectable bactericidal antibodies in serum frequently became carriers of the epidemic strain but did not develop meningococcal disease, while virtually all cases of disease occurred in the 18% of individuals whose baseline sera lacked bactericidal activity (titers of <1:4 measured with human complement). Recruits who lacked bactericidal antibodies and developed group C carriage had meningococcal disease attack rates as high as 38.5%.

Based on the Goldschneider et al. data, a bactericidal antibody titer of 1:4 or greater when measured with human complement has become an accepted surrogate of protective meningococcal immunity. However, the interpretation of a bactericidal titer of <1:4 as an indication of susceptibility is more problematic, and some have questioned whether the proportion of persons with titers below this threshold overestimates the size of the susceptible population (1).

In the present study, we used the infant rat bacteremia model to investigate the basis of naturally acquired immunity to N. meningitidis group C. Although it is impossible to be sure that protective activity measured by this animal model is entirely applicable to human disease, we found that adult sera with bactericidal titers of ≥1:4 were more likely to confer passive protective activity in the animal model than nonbactericidal sera (Table 3). Several sera with bactericidal titers of 1:4 or greater failed to confer protection, a result which may reflect some of the limitations of testing the passive protective activity of human antibody in the presence of infant rat complement and rat phagocytic cells and in an animal model that is subject to expected biologic variability. Nevertheless, the specificity of a bactericidal titer of 1:4 or greater for protective activity in the animal model was 91% when bactericidal activity was measured with internal human complement and 96% when measured with exogenous human complement (Table 4).

Approximately one-quarter of the nonbactericidal sera had passive protective activity in the animal model. These results are consistent with the conclusions of the 1969 Goldschneider et al. studies (10, 11) that the presence of serum bactericidal activity is a reliable marker of protection against disease but that the absence of serum bactericidal activity does not necessarily imply susceptibility, since not all recruits who lacked serum bactericidal activity and who became colonized with the epidemic strain developed invasive disease. Although not directly tested in our study, the most likely mechanism responsible for protective activity in the absence of serum bactericidal activity is opsonization (21), which can result from antibody binding to the bacterial surface and activation of C3b deposition without proceeding to bacteriolysis (36). Alternatively, there may be blocking of bactericidal antibodies in vitro, for example, by the presence of non-complement-activating IgA antibodies (14). In the infant rat, the IgA antibodies may be diluted to a concentration that does not block passive protective activity.

Protective antibodies to N. meningitidis group C strains can be directed at a number of antigenic targets, including the capsular polysaccharide or noncapsular antigens, such as PorA (18) or Opc (class 5 outer membrane proteins) (31). Based on absorption studies of gamma globulin prepared from more than 2,000 North American donors, Goldschneider et al. concluded that the majority of naturally acquired group C serum bactericidal activity was directed against the capsular polysaccharide (10), which is consistent with our absorption data on individual sera. Our results also showed a strong association between serum passive protective activity for the animal model and the presence of higher concentrations of naturally acquired group C anticapsular antibodies than anticapsular antibody concentrations in sera that failed to confer protection in the animal model (Table 2 and Fig. 2A). Our data also under-
score the importance of the quality of the anticapsular antibodies, since protective activity was associated with high-avidity anticapsular antibodies (Fig. 2B).

In the Goldschneider et al. studies, 82% of military recruits had group C meningococcal titers of ≥1:4 (10, 11). In contrast, only 20% of unimmunized adults in the present study had bactericidal titers of ≥1:4 when measured with human complement (Table 3), which was the complement source used in the Goldschneider et al. studies. A study performed in British Columbia from 1991 through 1993, which used group C strain C11 (the same strain as that used in the Goldschneider et al. studies), found that only 9.5% of adolescents, aged 13 to 19 years, had bactericidal titers of >1:4 (24). Recent studies in the United Kingdom also reported that only 10 to 30% of sera from unimmunized adults were positive for group C bactericidal antibodies (19, 34). Taken together, the data suggest that the seroprevalence of group C bactericidal activity has decreased in the population since the 1960s, when the Goldschneider et al. studies were done. A decrease in the prevalence of serum bactericidal antibodies in the adult population may explain the recent apparent increase in the rate of meningococcal disease in neonates in the United States (33), since newborns are thought to be protected from developing meningococcal disease by transplacentally acquired maternal antibodies (10, 28).

Group C polysaccharide-protein conjugate vaccines were introduced in the United Kingdom in the fall of 1999 and were followed soon thereafter by a marked decrease in the incidence of group C meningococcal disease by transplacentally acquired maternal antibodies. Conjugate vaccines are their ability to prime for immunological memory at age 4 years after meningococcal group C conjugate vaccination in children in the United Kingdom. J. Infect. Dis. 186:1353–1357.


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