Natural and Vaccine-Induced Immunity and Immunologic Memory to \textit{Neisseria meningitidis} Serogroup C in Young Adults

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The immune response to polysaccharides and conjugate vaccines in adults is poorly understood. This study assessed meningococcal serogroup C responses after AC polysaccharide (MACP) and C conjugate (MCC) vaccine administration in young adults and explored immune memory by measuring antibody avidity. The geometric mean avidity indices (GMAIs) measured 1 month after MACP vaccination were relatively high and failed to increase significantly in the 6 months before and after a second dose of MACP/MCC. Although the GMAI of naïve adults increased immediately following MCC vaccination to 215.7 (95% confidence interval, 181.0–257.1), a level similar to that seen after MACP vaccination, no further maturation in the subsequent 6 months was seen. Antibody induced by polysaccharide antigens in adults is already of relatively high avidity (compared with that in infants and toddlers) and fails to mature further, probably because both MACP and MCC predominantly stimulate memory B cells.

\textit{Streptococcus pneumoniae}, \textit{Neisseria meningitidis}, and some \textit{Haemophilus influenzae} strains are major human pathogens and cause a disproportionate amount of invasive disease in early life. Their success as pathogens is partly related to the poor immunogenicity of their polysaccharide capsules. Antibodies directed against capsules are protective but are difficult to induce in the first 18 months of life, partly because of the underlying mechanism by which the immune system handles polysaccharide antigens. Polysaccharides are handled as T-independent antigens and, apart from resulting in poor immunogenicity in the young, are also associated with the absence of the induction of memory cells. Thus, available capsular polysaccharide vaccines are unable to prevent disease in those persons most at risk.

The development of protein-polysaccharide conjugate vaccines has overcome these limitations by presenting the polysaccharide moiety of the vaccine covalently coupled to a carrier protein. Although the role of professional antigen-presenting cells is unclear, it is likely that B cells recognizing the polysaccharide process the protein carrier and present it to CD4$^+$ T cells. These carrier-specific T cells provide help to the B cell, resulting in increased immunogenicity to the polysaccharide component in those unresponsive to pure polysaccharide (e.g., infants) and in the generation of immune memory.

Little is understood of the immune response to polysaccharide antigens and conjugate vaccines in adults. Recent evidence suggests that memory B cells are responsible for the antibodies produced following the immunization of adults with \textit{H. influenzae} type b (Hib) conjugate or pneumococcal polysaccharide or conjugate vaccines [1, 2], but nothing is known of the meningococcal C response.

Antibody avidity, the strength with which bivalent antibody binds to complex antigens, has been used as a surrogate marker of memory and was recently applied to the study of conjugate vaccines [3–7]. In the current study, we studied young adults following \textit{N. meningitidis} serogroup C polysaccharide or conjugate vaccination, with the aim of establishing whether an encounter with such vaccines in adult life is associated with an increase in avidity over time, as seen in young infants and toddlers [7, 8], or whether an encounter with these vaccines may induce the characteristics of a memory response analogous to that recently described for Hib [1] and the pneumococci [2].

\textbf{Patients and Methods}

\textit{Study population.} The study population was composed of university students who had received a single dose of meningococcal AC polysaccharide (MACP) vaccine as part of outbreak control measures at Salford University (Salford, United Kingdom) in late 1997 [9]. They were randomized to be revaccinated with either MACP or meningococcal serogroup C conjugate (MCC) vaccine.
6 months later. A control group of unvaccinated Salford University students received a single dose of MCC vaccine when the study population received their second vaccine.

**Study vaccines.** MACP vaccine (MengiVac A+C; Pasteur Merieux) contained 50 μg each of serogroups A and C polysaccharides. MCC vaccine (Wyeth Lederle Vaccines) contained 10 μg of meningococcal C oligosaccharide linked to 15 μg of CRM197 mutant diphtheria toxoid. Both vaccines contained the O-acetylated form of meningococcal C polysaccharide and were administered by intramuscular injection in the arm.

**Serogroup C--specific IgG levels and serum bactericidal activity (SBA).** Meningococcal serogroup C--specific IgG concentrations were measured by a standardized ELISA as described elsewhere [10], with a standard reference serum (CDC1992; provided by G. Carlone, Centers for Disease Control and Prevention, Atlanta) assigned a value of 24.1 μg/mL for serogroup C--specific IgG [11]. The meningococcal C polysaccharide was O-acetylated (National Institute of Biological Standards and Control, Potters Bar, United Kingdom). The lower limit of antibody detection was 0.1 μg/mL; results below this limit were reported as 0.05 μg/mL for computational analyses. The SBA against C11 meningococcal strain (phenotype C:16:P1.7;1) used pooled baby rabbit serum (Pel-Freeze) as an exogenous complement source [9]. SBA titers were expressed as the reciprocal of the final serum dilution giving ≥50% killing at 60 min.

**Serogroup C--specific antibody avidity indices.** Avidity was measured by an elution ELISA incorporating thiocyanate and modified for meningococcal C as described elsewhere [5, 12]. Serum samples were diluted in buffer containing 10 mM PBS, 0.01% Brij 35, and 5% newborn bovine serum to a concentration of 0.5 μg/mL and were incubated on an antigen-coated plate overnight at 4°C. Plates were then washed, and ammonium thiocyanate, diluted in the serum buffer at varying concentrations (0–1 M), was added to the wells. After 15 min, the plates were washed and the antibody detected by using polyclonal horseradish peroxidase–labeled goat antibody to human IgG. The assay was developed by use of the chromogenic substrate 3,3,5,5-tetramethylbenzidine in citric acid–phosphate buffer and was stopped, after 30 min, with 2 M H2SO4. The absorbance was then read at 450 nm. The avidity index (AI) was derived by plotting the log of the percent reduction in the optical density against the thiocyanate concentration and calculating the amount of thiocyanate required to reduce the absorbance in a given serum sample by 50%. Meningococcal C IgG avidity was evaluated in subjects with antibody levels >1 μg/mL. An AI of 50 was considered to be the lower limit of the assay.

**Statistical analysis.** AIs were log-transformed, and geometric mean avidity indices (GMAs) were calculated with 95% confidence intervals (CIs). Differences between time points were analyzed by using paired t tests and differences between groups by unpaired t tests.

**Results**

Table 1 shows meningococcal serogroup C--specific IgG antibody levels, SBA titers, and GMAs. Six months after MACP vaccination, both serogroup C IgG and SBA levels fell significantly (P < .001) but remained much higher than the baseline levels seen in naive controls. Following the reimmunization with MACP, there was no significant increase from the 6-month post–initial vaccine level in SBA (P = .11) or IgG (P = .84), a hyporesponsiveness effect that was previously reported [9]. Reimmunization with MCC, however, resulted in significant increases (P < .001) in SBA and IgG levels, similar to those seen after initial vaccination. After MCC vaccination, the control group achieved SBA and IgG levels significantly higher (P < .001) than the group reimmunized with MACP, but they were not significantly different from those of the

<table>
<thead>
<tr>
<th>Study group, time point</th>
<th>No.</th>
<th>GMAI (95% CI)</th>
<th>No.</th>
<th>IgG GMC (95% CI)</th>
<th>No.</th>
<th>SBA GMT (95% CI)</th>
</tr>
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<tbody>
<tr>
<td><strong>2 MACPs</strong></td>
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<td></td>
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<tr>
<td>1 Month after first MACP vaccination</td>
<td>31</td>
<td>167.2 (129.6–215.8)</td>
<td>39</td>
<td>25.9 (17.6–38.2)</td>
<td>39</td>
<td>428.6 (205.0–896.2)</td>
</tr>
<tr>
<td>6 Months after first MACP vaccination</td>
<td>38</td>
<td>168.4 (132.5–213.9)</td>
<td>51</td>
<td>15.3 (10.7–21.8)</td>
<td>51</td>
<td>98.9 (54.4–179.7)</td>
</tr>
<tr>
<td>1 Month after second MACP vaccination</td>
<td>35</td>
<td>177.4 (140.5–224.1)</td>
<td>42</td>
<td>13.9 (9.4–20.5)</td>
<td>42</td>
<td>139.0 (72.5–266.5)</td>
</tr>
<tr>
<td>6 Months after second MACP vaccination</td>
<td>27</td>
<td>214.1 (165.1–277.7)</td>
<td>28</td>
<td>10.9 (6.3–18.8)</td>
<td>28</td>
<td>137.9 (50.8–374.2)</td>
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<tr>
<td><strong>MCC/MCC</strong></td>
<td></td>
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<tr>
<td>1 Month after first MACP vaccination</td>
<td>31</td>
<td>204.4 (157.6–264.9)</td>
<td>38</td>
<td>30.4 (20.4–45.4)</td>
<td>38</td>
<td>512.0 (233.8–1121.3)</td>
</tr>
<tr>
<td>6 Months after first MACP vaccination</td>
<td>37</td>
<td>180.2 (143.4–226.5)</td>
<td>48</td>
<td>26.6 (19.2–36.9)</td>
<td>48</td>
<td>93.2 (49.8–174.4)</td>
</tr>
<tr>
<td>1 Month after second MACP vaccination</td>
<td>35</td>
<td>212.0 (163.5–274.9)</td>
<td>41</td>
<td>41.1 (29.9–56.5)</td>
<td>41</td>
<td>454.9 (264.4–782.4)</td>
</tr>
<tr>
<td>6 Months after second MACP vaccination</td>
<td>27</td>
<td>182.4 (136.4–244.0)</td>
<td>28</td>
<td>18.6 (10.4–33.3)</td>
<td>28</td>
<td>475.4 (210.3–1074.6)</td>
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</table>

**NOTE.** CI, confidence interval; NA, not applicable.

* a Nos. relate to the values to the right.
* b Values for 18 of 32 adults were <50.
* c Values for 35 of 50 adults were <40.
group reimmunized with MCC vaccine ($P = .17$ for SBA; $P = .96$ for IgG).

GMAlis measured 1 month after vaccination with MACP remained stable 6 months later and failed to increase significantly immediately following and 6 months after a second dose of the vaccine, regardless of the type of vaccine (MACP or MCC) used. Of the 32 naive control subjects with preimmunization IgG levels >1 $\mu$g/mL, the AI of the majority was below the limit of detection; the GMAI of the rest was 180.3 (95% CI, 134.5–241.7). All control subjects had measurable AIs after MCC vaccination, and the GMAI was 215.7 (95% CI, 181.0–257.1). For the group with detectable avidity before immunization, the GMAI 1 month after immunization increased significantly to 300.8 (95% CI, 231.9–390.2; $P = .003$). Avidity then declined by an average of 20%.

### Discussion

We identified several important aspects of the immune response to meningococcal serogroup C polysaccharide in young adults. Antibody induced by MACP was of relatively high avidity 1 month after immunization and was similar to that 1 month after MCC administration. This contrasts with the relatively low IgG avidity observed following either MACP or MCC vaccination in infants and toddlers previously studied in our laboratory [6, 8]. As expected, no increase in avidity was detected in the 6 months after MACP vaccination, but, unexpectedly, no increase was detected following MCC vaccination of previous MACP recipients. Although avidity increased 1 month after MCC administration in naive recipients, there was no further increase in avidity in the following 6 months. This is in marked contrast to the experience with MCC vaccines [7, 8] and Hib [2, 13] and pneumococcal conjugate vaccines [4] administered to infants and toddlers in whom avidity continued to increase in the months following primary immunization.

High-avidity antibody produced after a single dose of plain polysaccharide or conjugate vaccine would be consistent with an antibody response dominated by memory B cells. In this respect, our data support those recently generated by the study of memory B cells at the molecular level following immunization of healthy adults with polysaccharides. Hougs et al. [1] analyzed variable-region gene rearrangements by single-cell polymerase chain reaction of Hib-specific B cells following a challenge of Hib polysaccharide (polyribosyl ribitolphosphate [PRP]) conjugate in a vaccine-naive adult. The PRP-specific B cells isolated from peripheral blood 7 days after immunization showed characteristic gene rearrangements, indicating dominance by memory B cells. One of us (D.G.) recently reported the results of molecular analysis of hybridomas generated from the blood of adult pneumococcal vaccine recipients [2]. These results demonstrated that B cells isolated from the peripheral blood of pneumococcal plain polysaccharide or conjugate recipients 7 days after immunization were dominated by memory B cells.

Molecular analysis of meningococcal serogroup C–specific B cells would provide an opportunity to obtain definitive evidence of the nature of B cells stimulated by meningococcal vaccine in adults. The absence of further avidity maturation in the months after conjugate vaccination would suggest that the memory B cells stimulated by the vaccine may have achieved maximal somatic hypermutation, although the mechanism for defining this threshold is unclear. Further somatic mutation may be associated with a loss of antigen-binding specificity.

Although purified capsular polysaccharides are T-independent antigens and vaccination with such antigens is not associated with the induction of immunologic memory, it seems likely that the exposure to capsular polysaccharides, in the context of live bacteria (perhaps during nasopharyngeal colonization) or cross-reacting antigen over several years, is a priming event. Priming may occur because capsular polysaccharide on the surface of a bacterium may be seen by the immune system as a molecule conjugated to outer-membrane proteins. Natural priming might partly explain the decline in the incidence of invasive infection due to encapsulated bacteria beyond early childhood.

Our study confirmed the presence of immunologic hyporesponsiveness after a second dose of MACP vaccine, and it also confirmed that MCC vaccination can overcome this hyporesponsiveness [9]. Of interest, the avidity achieved 1 month after a second dose of MACP vaccine was similar to that seen 1 month after MCC vaccination in both vaccine-naive adults and in those who had previously received MACP vaccine. This suggests, that despite a reduced antibody level observed after a second dose of MACP vaccine, the B cells being stimulated are qualitatively competent. The mechanism responsible for the observed hyporesponsiveness remains poorly understood but may be due to the depletion of the available meningococcal C polysaccharide–reactive B cell pool by MACP vaccine. This vaccine clearly can stimulate, but not regenerate, memory B cells (which become plasma cells and then die).

Avidity maturation is gaining acceptance as a tool for study of the generation of memory cells following conjugate vaccination in infants and toddlers. We hoped this might prove to be a similarly useful tool in adults. We previously found in a small study the suggestion of a modest increase in avidity in the months following vaccination with a meningococcal C tetanus conjugate, but only a limited number of vaccine recipients were studied [6]. Konradsen [14] described the absence of avidity maturation in elderly recipients of plain pneumococcal polysaccharide vaccines over a 5-year period, whereas Wuorimaa et al. [15] recently demonstrated the absence of avidity maturation in adults following pneumococcal conjugate vaccination. These findings are consistent with those in our present study.

It is unclear why antibody avidity prior to immunization in the majority of naive vaccine recipients of MCC was low if the recipients had been primed as discussed above. One explanation is that
the primed B cells are relatively dormant, perhaps as a consequence of the route of priming (mucosal), and that the antibody measured in the serum of vaccine-naive persons is produced by plasma cells induced by cross-reactive antigens that are of low affinity. Antibody avidity is a useful tool for studying the development of immune memory to polysaccharide antigens in young recipients of conjugate vaccines. In adults, despite the advantage of conjugate vaccines over polysaccharide vaccines, avidity cannot be used to monitor the generation of immune memory, although avidity assays are a useful tool for helping to understand polysaccharide immunity in adults.

Acknowledgment

We thank Nick Andrews (Public Health Laboratory Service, Communicable Disease Surveillance Centre, London) for advice and for statistical analysis.

References