Antibody Persistence and Immunological Memory at Age 4 Years after Meningococcal Group C Conjugate Vaccination in Children in the United Kingdom

Ray Borrow,1 David Goldblatt,2 Nick Andrews,3 Jo Southern,3 Lindsey Ashton,2 Sarah Deane,1 Rhonwen Morris,1 Keith Cartwright,4 and Elizabeth Miller3

1Public Health Laboratory Service Meningococcal Reference Unit, Withington Hospital, Manchester; 2Immunobiology Unit, Institute of Child Health, and 3Immunisation Division, Public Health Laboratory Service Communicable Disease Surveillance Centre, London, and 4Public Health Laboratory, Gloucester Royal Hospital, Gloucester, United Kingdom

Antibody persistence and immunological priming for 2 formulations of a meningococcal group C (menC) conjugate (MCC) vaccine (containing 2 or 10 μg of menC polysaccharide) administered at 2, 3, and 4 months of age was investigated by boosting vaccine recipients at age 13–16 months or 4 years with 10 μg of unconjugated menC polysaccharide. At age 4 years, geometric mean titers (GMTs) and concentrations of menC-specific immunoglobulin G and serum bactericidal antibody (SBA) had decreased to prevaccination levels. Geometric mean avidity indices increased after the primary vaccination until age 13–16 months and then remained constant until age 4 years. One month after boosting at age 4 years, menC immunoglobulin G and SBA levels increased significantly. The postbooster SBA GMT for the 2-μg vaccination (2181.2; 95% confidence interval [CI], 975.9–4875.1) was 2-fold higher than that for the 10-μg vaccination (931.6; 95% CI, 338.0–2568.1). This is the first demonstration of immunological memory at 4 years of age in children receiving MCC vaccine on the United Kingdom’s 2/3/4-month immunization schedule.

Meningococcal serogroup C (menC) conjugate (MCC) vaccines were introduced in the United Kingdom in November 1999 for all children <18 years of age [1]. Although short-term serological correlates of protection are now established in the United Kingdom [2], which of the parameters measured in the serum immediately after primary immunization might predict long-term protection is not known. Long-term protection after natural infection or vaccination is related to the presence of immunological memory. Experience with MCC vaccines has already shown that serum antibody levels decrease rapidly within 12 months after primary immunization [3]. However, it is anticipated that MCC vaccines, like other, similar vaccines (e.g., bacterial polysaccharide glycoconjugates), will provide long-term protection, despite low antibody levels, and that this will be mediated via immunological memory. The ability to predict the generation of immunological memory after MCC vaccination is thus of particular importance.

Currently, we accept the demonstration of a booster response years after vaccination or an increase in avidity over time as indicative of the presence of immunological memory and, presumably, protection. The absence of a currently validated threshold predicting long-term protection makes it difficult to specify equivalence criteria between MCC and meningococcal sero-group A/C polysaccharide (MACP) vaccines. Moreover, the evidence available to date shows that the quantity of antigen used for the primary series is positively correlated with post–primary vaccination serum bactericidal antibody (SBA) levels but negatively correlated with the magnitude of booster response [3–5]. However, whether this dose-response effect is still apparent 4 years after the primary vaccination series is not known. No data are available, to our knowledge, on the persistence of immunological memory responses in infants in the United Kingdom who have received the 2/3/4-month vaccination schedule. The ability of 2 different formulations (2 and 10 μg) of MCC vaccine to prime for immunological memory was investigated by measurement of antibody concentrations and avidity indices after a 10-μg MACP challenge at 13–16 months or 4 years of age.

Subjects and Methods

Study population. The study population has been described elsewhere [3]; infants eligible for routine primary immunizations with diphtheria-tetanus toxoids–pertussis, Haemophilus influenzae
type b, and oral polio vaccines were recruited between October 1995 and March 1996 from general practices in west Gloucestershire. Infants were excluded if they were immunocompromised or if there was a general contraindication to immunization.

Vaccines and immunization. Two formulations of the MCC vaccine (Wyeth-Lederle Vaccines), containing either 2 or 10 μg of menC oligosaccharide, were used for primary immunization in sequential cohorts of infants, as reported elsewhere [3]. Approximately one-half of the infants were randomly assigned to receive a challenge dose of 10 μg of unconjugated menC polysaccharide (contained in an MACP vaccine [Meningivac, Pasteur Merieux]) at 13–16 months of age; the other half of the infants received the challenge dose at 4 years of age. The first 2 infants received a full dose of MACP vaccine, but, after adverse events were seen in 1 infant and reported in 3 other children receiving this vaccine in another study [3], the booster dose was lowered to 10 μg.

Blood samples were obtained from each infant by venipuncture before the first immunization, 4 weeks after each dose of vaccine, and at 14 months of age. Blood samples were also collected 1 month after boosting from children assigned to receive the booster dose at 13–16 months of age and before and 1 month after boosting for those assigned to receive the booster dose at 4 years of age.

Serological studies. Serum samples were tested for menC-specific IgG antibodies by ELISA and by bactericidal assays (incorporating rabbit complement), using standardized protocols, as described elsewhere [3]. menC-specific IgG avidity indices were measured as described elsewhere [6]. SBA titers were expressed as the reciprocal of the final serum dilution yielding 50% killing at 60 min.

Statistical analysis. Geometric mean concentrations (GMCs), titers (GMTs), and avidity indices (GMAIs) were calculated for each sampling time and group (2 or 10 μg), as were 95% confidence intervals. Differences between groups were assessed using unpaired t tests, and differences between sampling times were assessed using paired t tests. Significance was taken at a 5% level.

Results

Priming and antibody persistence data for menC-specific IgG GMCs, SBA GMTs, and GMAIs for both the low- and the high-dose formulation of the MCC vaccine are given in table 1. Mean menC-specific IgG and SBA levels at 4 years of age for both formulations had returned to prebooster levels. Only 8% (2 of 25) and 12% (3 of 25) of subjects for the low- and high-dose formulations, respectively, had SBA titers >8 (for the low-dose formulation), SBA titers for both patients were 8, whereas for the high-dose formulation, titers were 8, 64, and 1024. SBA GMTs increased after administration of a booster at 4 years of age; the level in subjects primed with the 2-μg MCC vaccine (2181.2) was 2.3-fold higher than the level in those primed with the 10-μg MCC vaccine (931.6) (table 2). This difference was not significant (P = .18). menC-specific IgG levels were similar for both formulations after boosting at 4 years of age (11.4 and 8.0 μg/mL for the low- and high-dose formulations, respectively) (table 2). Avidity indices were not measurable for the majority of infants before vaccination, because 78% (76 of 97) infants had IgG levels <0.5 μg/mL, the cutoff point for the assay.

After the first dose of MCC vaccine, the menC GMAIs were 81.3 and 72.7 among 2-μg and 10-μg MCC vaccine recipients, respectively. GMAIs increased sequentially after each dose and, by 9 months after the third dose, had risen significantly (P < .01), to 125.6 in the low-dose group and 114.7 in the high-dose group (table 1). GMAIs at 4 years of age remained at levels similar to those at 9 months after the third dose for the 16 individuals with measurable avidity (i.e., IgG level ≥0.5 μg/mL) (table 2). After administration of the MACP booster at 4 years of age, GMAIs were significantly higher (1.33 times higher in low-dose and high-dose groups combined; P < .01) than at 9 months after the third dose, but, because of the small number of subjects with available results, the increase between prebooster and postbooster levels was not significant for those who received the booster at 4 years of age (1.30fold increase for low- and high-dose groups combined; P = .010) (table 2). The low- and high-dose groups did not show any significant differences in GMAI at any time point.

Discussion

At 4 years of age, children in the United Kingdom who were immunized with the MCC vaccine at 2, 3, and 4 months have both menC-specific IgG GMCs and SBA GMTs similar to those found before vaccination, at 2 months of age. Despite the low titers of antibody, immunological memory was demonstrated by high SBA and menC-specific IgG levels achieved 1 month after a booster dose of plain polysaccharide and by the demonstration of a sustained increase in avidity. After administration of a booster dose of 10 μg of unconjugated menC polysaccharide at 4 years of age, both functional (SBA) and avidity indices were higher (P < .01) than in historical controls (infants ≥24 months of age) who received a full dose (50 μg) of meningococcal polysaccharide. These control children, who previously were naive to meningococcal vaccination, had SBA GMTs of 13.3 (95% confidence interval [CI], 4.7–37.4) and GMAIs of 101.0 (95% CI, 83.5–122.1) 1 month after vaccination with a full dose of polysaccharide [7].

Antibody avidity has been used as a surrogate marker of immunological memory, because changes in affinity over time are characteristic seen after vaccination with T cell–dependent antigens and rapid production of high-affinity responses is seen after boosting with such antigens. Measurement of avidity has been applied to studies of various conjugate vaccines, including those for menC [6, 8] and meningococcal group A [9], H. influenzae type b [10] and Streptococcus pneumoniae [11]. True validation of these measurements as correlates of immunity will emerge as the ongoing monitoring of the efficacy of the MCC vaccine introduced in the United Kingdom in 1999 continues [1].

Two other studies have addressed the question of whether long-term immunological memory results from administration
Table 1. Meningococcal group C (menC)–specific antibody concentrations before and 1 month after menC conjugate vaccination at 2, 3, and 4 months of age and antibody persistence at 13 to 16 months and 4 years of age, according to vaccine formulation.

<table>
<thead>
<tr>
<th>Dose, measure</th>
<th>Before vaccination</th>
<th>One month after first dose</th>
<th>One month after second dose</th>
<th>One month after third dose</th>
<th>Age 13–16 months</th>
<th>Age 4 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antibody concentration (95% CI)</td>
<td>No. of subjects with results</td>
<td>Antibody concentration (95% CI)</td>
<td>No. of subjects with results</td>
<td>Antibody concentration (95% CI)</td>
<td>No. of subjects with results</td>
</tr>
<tr>
<td>2 µg</td>
<td>SBA GMT menC-specific IgG GMC, µg/mL</td>
<td>2.1 (2.0–2.2)</td>
<td>48</td>
<td>29.8 (17.5–50.7)</td>
<td>48</td>
<td>496.8 (324.8–760.0)</td>
</tr>
<tr>
<td></td>
<td>GMAI</td>
<td>110.7 (72.7–168.6)</td>
<td>9</td>
<td>81.3 (73.6–89.7)</td>
<td>46</td>
<td>96.6 (90.8–102.8)</td>
</tr>
<tr>
<td>10 µg</td>
<td>SBA GMT menC-specific IgG GMC, µg/mL</td>
<td>2.4 (2.0–2.8)</td>
<td>51</td>
<td>29.7 (16.7–52.8)</td>
<td>47</td>
<td>724.1 (472.7–1109.1)</td>
</tr>
<tr>
<td></td>
<td>GMAI</td>
<td>76.5 (51.8–112.9)</td>
<td>12</td>
<td>72.7 (66.2–79.8)</td>
<td>42</td>
<td>98.5 (92.7–104.6)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; GMAI, geometric mean avidity index; GMC, geometric mean concentration; GMT, geometric mean titer; SBA, serum bactericidal antibody.

a No. of subjects for whom results of a particular test were available. Avidity indices could not be determined for subjects with menC-specific IgG levels $<0.5$ µg/mL.

b For SBA titer, the cutoff point was 1:8; for measurement of geometric avidity indices, the cutoff point was 0.5 µg/mL.
of a primary MCC series in infancy [12, 13]. However, these studies differed from ours, most significantly because both delivered an early booster after primary immunization; however, they also differed in the population studied, the vaccine schedule used, and the source of the vaccine. In a study of Gambian children, 1–3 doses of a bivalent A/C conjugate vaccine were administered in infancy, with a booster dose of the same conjugate vaccine administered at age 18–24 months [12]. The SBA GMT (measured using rabbit complement) after boosting with meningococcal A/C polysaccharide vaccine, according to primary vaccine formulation.

Table 2. Meningococcal group C (menC)–specific antibody concentrations before and 1 month after boosting with meningococcal A/C polysaccharide vaccine, according to primary vaccine formulation.

<table>
<thead>
<tr>
<th>Dose, measure, subject age at boost</th>
<th>Before administration of booster</th>
<th>One month after administration of booster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antibody concentration (95% CI)</td>
<td>No. of subjects with results</td>
</tr>
<tr>
<td>SBA GMT</td>
<td></td>
<td>All&lt;sup&gt;b&lt;/sup&gt; Below cutoff&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>13–16 months</td>
<td>26.3 (11.5–59.8)</td>
<td>21 7 1066.6 (498.4–2282.8) 17 0</td>
</tr>
<tr>
<td>4 years</td>
<td>2.6 (2.1–3.1)</td>
<td>25 23 2181.2 (975.9–4875.1) 22 0</td>
</tr>
<tr>
<td>menC–specific IgG GMC, μg/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–16 months</td>
<td>0.79 (0.57–1.10)</td>
<td>21 — 19.0 (12.7–28.5) 18 —</td>
</tr>
<tr>
<td>4 years</td>
<td>0.25 (0.14–0.42)</td>
<td>25 — 11.4 (7.9–16.3) 23 —</td>
</tr>
<tr>
<td>GMAI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13–16 months</td>
<td>122.5 (104.1–144.1)</td>
<td>15 5 160.5 (137.9–186.8) 18 0</td>
</tr>
<tr>
<td>4 years</td>
<td>130.3 (80.4–211.0)</td>
<td>9 14 170.5 (150.6–193.0) 22 0</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; GMAI, geometric mean avidity index; GMC, geometric mean concentration; GMT, geometric mean titer; SBA, serum bactericidal antibody titer.

<sup>a</sup> No. of subjects for whom results of a particular test were available. Avidity indices could not be determined for subjects with menC-specific IgG levels <0.5 μg/mL.

<sup>b</sup> For SBA titer, the cutoff point was 1:8; for measurement of geometric avidity indices, the cutoff point was 0.5 μg/mL.

SBA GMTs at 1 month after boosting were higher for the 2-μg than for the 10-μg formulation, although this difference did not achieve statistical significance. SBA GMTs at 4 years of age for the 2-μg formulation were 2-fold higher than those for the 10-μg formulation. The inverse relationship between the amount of antigen in the vaccine and the magnitude of memory response was first noted in those boosted 9 months after primary immunization [3] and is now seen to persist to the age of 4 years. This phenomenon has been reported for other meningococcal [4] and pneumococcal [5] conjugates.

Although the antibody titers varied according to vaccine formulation, the avidity indices were not significantly different. For both formulations, avidity increased significantly through the primary series to 14 months of age and then further increased after boosting.

This is the first report of antibody persistence and induction of immunological memory in 4-year-old children immunized in the United Kingdom with the 2/3/4-month vaccination schedule. These immunological data are encouraging and suggest that primary immunization with a conjugate vaccine on an accelerated schedule is able to prime efficiently for immunological memory responses later in life. These data are highly relevant for countries that use the Expanded Programme on Immunization schedule, which makes no provision for receipt of booster vaccines in the second year of life and which may incorporate conjugate vaccines.
in the future. It is also relevant to the efforts to develop and implement meningococcal group A conjugate vaccines for the “meningitis belt” in sub-Saharan Africa. It may be possible to provide long-term immunity against meningococcal group A with only 1 or 3 doses of a conjugate vaccine and thus provide the type of protection required by countries where epidemics may occur regularly and where repeated access to vaccinees may be impractical.

References