Kinetics of Immune Responses to Nasal Challenge With Meningococcal Polysaccharide One Year After Serogroup-C Glycoconjugate Vaccination

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Background. Recipients of serogroup-C glycoconjugate meningococcal vaccine (MCC) exhibit waning of serum bactericidal antibody (SBA) titers, but the rate of decline and the speed of their immunological memory in response to new meningococcal nasopharyngeal colonization are unknown.

Methods. In a prospective challenge study, we measured persistence of SBA and anti–Neisseria meningitidis serogroup-C (MenC) immunoglobulin (Ig) G and IgA in adults aged 18–39, 28 days and 12 months after receiving MCC. Volunteers were then challenged intranasally with 50 μg MenC polysaccharide to mimic meningococcal colonization, and systemic and mucosal antibody responses were measured.

Results. All subjects had protective SBA titers (≥8) 28 days after MCC vaccination, but 12.3% and 20.2% had unprotective (<8) or low (<128) levels, respectively, after 12 months. Following rechallenge (12 months postvaccination) and measurement of antibody responses after 4, 7, and 10 days, rises in SBA titers were only observed in subjects with low (<128) or nonprotective (<8) prerechallenge SBA titers. In subjects with prerechallenge SBA titers <8, the majority did not reach a protective SBA titer until 7 days post-rechallenge. MenC-specific IgG levels rose in both serum and saliva in correlation with SBA titers. No detectable rise in salivary IgA was observed.

Conclusions. In those individuals who fail to retain protective SBA 12 months after MCC, immunological memory fails to generate protective systemic and mucosal antibodies until 7 days post intranasal challenge with cognate meningococcal polysaccharide. This is likely too slow to protect from natural meningococcal infection. MCC vaccinees rely on persistence of antibody levels rather than immunological memory for sustained protection.
colony could therefore protect some individuals with subprotective antibody titers in the time between colonization and invasive disease, if such a response was induced and was sufficiently rapid. Therefore, an important public health question concerns the speed of protective immune responses induced by immunological memory in vaccine recipients, in the event that their nasopharynx becomes colonized by *N. meningitidis*. In this study we have measured the persistence of antibodies to serogroup-C *N. meningitidis* 1 year after vaccination of young adults with MCC and investigated the kinetics of both the systemic and mucosal immune response to intranasal challenge with serogroup-C meningococcal polysaccharide, as a model of meningococcal colonization 1 year after receipt of MCC.

**METHODS**

**Study Population and Clinical Procedures**

In total, 116 undergraduate and postgraduate students (median age, 23; range, 19–39 years) presenting for meningococcal vaccination were vaccinated with MCC conjugated to tetanus toxoid (NeisVac-C, Baxter). Students attended for vaccination because they had not previously received MCC or plain polysaccharide vaccination. Antibody responses were measured in serum taken at 0 and 28 days and again 1 year later. At the 12-month postvaccination visit, subjects were challenged with nasal administration of 50 μg MenC polysaccharide vaccine (ACWY Vax, GSK). This was inoculated with the volunteers in the supine position with the neck extended and tilted down; then 5 mL of saline containing 25 μg of serogroup-C polysaccharide was inoculated into each nostril. Of the original 116 subjects, 89 gave consent for the intranasal challenge. Serological responses in blood and saliva were assessed at 0, 4, 7, and 10 days postchallenge. ORACOL (Malvern Medical Supplies) devices were used for saliva collection. Saliva was extracted from oral swabs by centrifugation in transport buffer at 2000 g (5 minutes), and aliquots were stored at −80°C. Blood samples were centrifuged for serum separation and stored at −20°C. All subjects gave informed consent, and the procedures were approved by the National Research Ethics Service (United Kingdom, Ref. 04/S0501/34).

**Serology and Saliva Analysis**

Serum samples were assessed by MenC SBA assay as described previously [8], with baby rabbit serum (Pel-Freez Inc) as an exogenous complement source. The strain used was C11 (C16:PL1.7-1,1). SBA titers were expressed as the reciprocal of the final serum dilution giving ≥50% killing after 60 minutes. For computational purposes SBA titers <4 were assigned a value of 2.

Total immunoglobulin (Ig) G and IgA levels from saliva samples were assessed by cytometric bead array Flexsets (BD) according to the manufacturer’s instructions. Results were collected on a BD FACSArray using BD FACSArray software for acquisition and FCAP Array software (Soft Flow) for analysis. MenC-specific IgG in serum and both IgG and IgA concentrations in saliva were determined using a modification of the serum IgG multiplex bead assay previously described [9].

To control for variation in saliva collection saliva results are expressed as ng specific antibody/μg total antibody. Total and specific Ig concentrations can be found in supplemental tables 1 and 2.

**Analyses**

A rabbit SBA (rSBA) titer of ≥8 has previously been demonstrated to be correlated with short-term clinical protection [7]. An rSBA titer of ≥128 predicts a human SBA titer (derived using human as opposed to rabbit complement) of at least 4, and correlates of protection identified by Goldschneider and colleagues [10] and which has been proposed as a more stringent measurement of vaccine protection [11]. However, more recent data have suggested that this cutoff titer of 128 may be unnecessarily stringent [1, 12]. In this study we have classified volunteer groups as SBA unprotected (SBA <8), SBA low (SBA <128), and SBA high/protected (SBA ≥128) for reporting of the serological data.

**Statistics**

Distribution of results was analyzed graphically and by the D’Agostino-Pearson test to establish if results had a parametric distribution. For multiple comparisons, where data were not normally distributed, a Friedman test was used. In order to reduce the potential for type II errors induced by multiplicity corrections, we restricted statistical analysis to relevant comparisons by use of a Dunn selected-pairs posttest. Correlations were carried out by Spearman nonparametric correlation. All statistics were performed on GraphPad Prism software (GraphPad).

**RESULTS**

**Responses to Initial Vaccination**

The majority of subjects had either unprotective or low SBA levels prevaccination (70 and 80%, respectively; Table 1). Twenty-eight days following vaccination no subjects had unprotective SBA titers, while only one subject had a low SBA titer.

A moderate positive correlation between the MenC-specific IgG concentrations and SBA titers was found both pre- and postvaccination (Table 2). Exactly 1 year following vaccination, a number of donors had dropped into the unprotected and low SBA groups (12.3 and 20.2%, respectively; Table 3).
Table 1. MenC SBA GMTs (95% CI) and Percentages of Subjects With MenC SBA Titers <8 and <128 Pre- and Postvaccination

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<tr>
<th>Parameter</th>
<th>Days after vaccination</th>
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<tbody>
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<td></td>
<td>0</td>
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<tr>
<td>No. of subjects</td>
<td>116</td>
</tr>
<tr>
<td>SBA GMT (95% CI)</td>
<td>10.5 (6.6–16.9)</td>
</tr>
<tr>
<td>% of subjects with SBA titer &lt;8</td>
<td>70.6 (82/116)</td>
</tr>
<tr>
<td>% of subjects with SBA titer &lt;128</td>
<td>80.17 (93/116)</td>
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Individuals With SBA Titers <8 or ≥8 to <128 1 Year Postvaccination Respond to Nasal Challenge With MenC Polysaccharide, but Subjects With an SBA Titer ≥128 Exhibit No Response

One year after the initial vaccination, study participants were rechallenged with nasal administration of meningococcal polysaccharide. Following rechallenge with polysaccharide, the majority of subjects were able to mount a response and generate protective SBA titers by 10 days, although most (9 of 11) of the donors with initial SBA titers below 8 had failed to reach protective levels 4 days after rechallenge.

Although a rise over time in the SBA geometric mean titers (GMTs) could be seen after rechallenge (Table 3), the observed rise in SBA GMT occurred only in the SBA unprotected group (Figure 1A–B). This rise was only statistically significant at 10 days post-rechallenge in the SBA unprotected group (P < .01, Friedman test with Dunn selected pairs posttest, day 0 versus 10). In subjects with pre-rechallenge SBA titers <8 the majority did not reach a protective SBA titer until 7 days post-rechallenge. GMTs in the SBA high group remain largely unchanged (Figure 1C).

Among those subjects with unprotective SBAs 1 year after vaccination, 91% (10 of 11) had a pre-vaccination SBA titer of <8 (the other had an SBA titer of 8), while 86% (6 of 7) and 60% (28 of 71) of subjects with low or high SBA levels 1 year after vaccination initially had SBA titers <8 (unprotected versus high SBA, P = .004; low versus high, P = .05, Yates corrected χ²; unprotected versus low, not significant).

Serum IgG Concentrations Are Positively Correlated With SBA Titers Following Rechallenge

Serum IgG concentrations rose following rechallenge and displayed moderate positive correlations to SBA titers at all time points (Table 3).

Salivary IgG Concentrations Rise on Rechallenge While IgA Levels Do Not

Following rechallenge no rise in the concentration of salivary IgA was observed by 7 days postchallenge; in addition, IgA levels did not display a significant correlation with SBA titers or either serum of salivary IgG (Table 4). Salivary IgG levels showed a moderate rise by day 7, were moderately correlated with SBA titers, and were strongly correlated with serum IgG concentrations (Table 5). Total and specific salivary IgA and IgG concentrations can be found in supplemental tables 1 and 2, respectively.

**DISCUSSION**

We have found that recall immune response to polysaccharide antigen that enters the nasopharynx may be too slow (>7 days) for clinically relevant protection even in recent recipients of glycoconjugate vaccine. In this prospective study we have shown that 100% of subjects developed a protective immune response to serogroup-C N. meningitidis 28 days after MCC vaccination but that 12% −20% of recipients exhibited waning of SBAs to unprotective/low levels by 1 year later. Among those individuals whose SBA levels are unprotective after 1 year, the majority can generate a protective response to intranasal challenge with serogroup-C polysaccharide, but this is significant only after 10 days following challenge, and the majority are unprotected after 4 days postchallenge. This is an important observation, because it is likely that such individuals will not be protected by a memory response to capsular polysaccharide if they become colonized by virulent N. meningitidis, despite receiving cognate glycoconjugate vaccine 1 year prior.

From a public health perspective, this study reveals that in the event of emergence of meningococcal disease in a community of young adults, a significant proportion (at least 20%) may be susceptible to invasive disease even if they have received cognate glycoconjugate vaccine more than 1 year prior. Vaccinees who become colonized will mount an immune response, but this is unlikely to be fast enough to prevent disease in those with low SBA titers at the point of acquisition.

Our observation that 12% had lost protective levels of SBA by 1 year after MCC is less than the 40% loss of protection seen within 2 years postvaccination in 15–17 year olds vaccinated as...
part of the UK vaccination campaign [13]. This may be due to the older cohort used here and the earlier sampling point used in this study (1 year versus within 2 years). Furthermore, in the current study, we used NeisVac-C which is more immunogenic and has higher antibody persistence than other MCC vaccines used for the majority of the reported catch-up vaccination campaign [14].

The kinetics of the serum response to nasal challenge are even more sluggish than those reported previously by Snape et al [15], who found that after challenge of immunized 13–15 year olds with intramuscular meningococcal polysaccharide, 3 years after receiving glycoconjugate vaccine, there was no increase in SBA observed until day 5 after revaccination. The difference in the current study is that intranasal polysaccharide was used as the rechallenge to mimic natural colonization by \textit{N. meningitidis} carrying the same polysaccharide present in the vaccine. We argue that if community vaccinees are exposed to serogroup-C \textit{N. meningitidis} and respond with similar kinetics to the donors investigated in this study, then it is likely they would not be able to mount a memory response quickly enough for protection and should be considered vulnerable to secondary vaccine failure. A number of commentators through the early 20th century (quoted by Goldschnieder and colleagues [10]) observed that in natural meningococcal infection there is a relatively short period between initial colonization and disease, and more recently investigators of outbreaks of serogroup C disease have reported short intervals (3–7 days) between exposure and disease [16, 17]. However, there has been at least one reported case of a delay of many weeks between acquisition of carriage of \textit{N. meningitidis} (serogroup B) and occurrence of disease [18], so it may not be the case that all disease occurs shortly after acquisition. It has been suggested that persistence of antibody may be a more appropriate measurement of long-term protection than immunological memory to capsular polysaccharide [6, 15], and these results support that conclusion.

We saw no significant rise in the SBA titers of individuals who, immediately prior to polysaccharide rechallenge, had SBA titers >128. There are several potential explanations for this. The phenomenon of high antibody levels blocking later antibody responses to specific antigens has been known for some time [19]. Several mechanisms have been described to account for this effect [20]. High levels of antibody binding may mask the epitope by blocking the interaction between the epitope and the B cell receptor by steric hindrance. Another possibility is that antibody binding leads to rapid clearance of the antigen, reducing the availability of polysaccharide for B cell recognition. Recent evidence suggests that epitope masking may be the dominant mechanism [21].

As a result, it is possible that the higher level of salivary IgG seen in SBA-protected donors may be sufficient to saturate and mask the available polysaccharide epitope before an antibody response can be mounted; however, since the level of polysaccharide used was quite high (50 \mu g) it does not seem likely that this would be the case. An alternate possibility is that a small systemic response occurs but the SBA assay itself is not sensitive enough to detect any change in donors with high initial SBA levels, particularly since it can only detect a minimum of a 2-fold change.

In this study we found no evidence of a rise in salivary IgA levels following rechallenge with polysaccharide or any correlation with SBA or IgG (in both serum and saliva). Salivary IgA was quite high (50 \mu g/mL) the day after rechallenge and was strongly correlated with serum IgG concentrations. Given the lack of a response in salivary IgA and the close correlation between salivary IgG and serum IgG, it seems likely that the immune response to nasal polysaccharide challenge elicits a systemic but not mucosal antibody response, with the observed increase in salivary IgG being due to transudation of serum IgG into the saliva. This is in agreement with several previous studies that have suggested that salivary IgG is mostly serum derived [22–24], although these studies used intramuscular injection rather than the nasal administration of polysaccharide performed here.

Robinson and colleagues previously found some evidence of rises in salivary \textit{N. meningitidis} serogroup-B (MenB)–specific IgA and IgG in university students in response to natural colonization by MenB; however, these were

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<tr>
<td>No. of subjects</td>
<td>89</td>
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<tr>
<td>SBA GMT (95% CI)</td>
<td>549 (315–955)</td>
</tr>
<tr>
<td>% of subjects with SBA titers &lt;8</td>
<td>12.3 (11/89)</td>
</tr>
<tr>
<td>% of subjects with SBA titers &lt;128</td>
<td>20.2 (18/89)</td>
</tr>
<tr>
<td>IgG ng/mL GMC (95% CI)</td>
<td>6,794 (4,489–10,285)</td>
</tr>
<tr>
<td>Spearman (r) (95% CI) with SBA titers</td>
<td>.64 (.49–.75)</td>
</tr>
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nonsignificant due to intersubject variability [25]. Since recurrent nasopharyngeal carriage of MenC occurs in the population [26], it was likely that a proportion of the tested donors would have protective SBA titers before initial vaccination. In the current study, only 9% of subjects who lost protection after one year had SBA levels $\geq 8$ pre-vaccination compared with 40% of subjects with SBA titers $\geq 128$ one year postvaccination. This may indicate that the SBA low group may be poor at maintaining immunity not only after vaccination but also following natural exposure to meningococci. We have previously found that individuals with serogroup C disease despite MCC vaccination exhibit reduced ex vivo T cell responses to polyclonal activators long after convalescence has occurred, suggesting that an intrinsic immune defect is present in “vaccine failures” [27]. Furthermore, there is an apparent intrinsic defect of B cell responsiveness to T cell–independent type II antigens in nonvaccinated adults who have experienced serogroup C disease [28]. The finding that 91% of subjects whose SBA titers had dropped below 8 one year after vaccination were able to mount an immune response to meningococcal polysaccharide demonstrates an anamnestic response, as this group is still able to mount a memory response on rechallenge.

One reservation that should be attached to our study concerns the assumption that polysaccharide inoculated into the nasopharynx mimics the natural challenge of live bacteria achieving colonization of this niche. In the latter case, there is replication of the organism and sustained interaction with the mucosa, which results in exposure of the host to multiple immunologically relevant bacterial components aside from polysaccharide. We recently reported that inoculation of live Neisseria lactamica, an organism without a polysaccharide capsule, into the human nasopharynx induces a specific serum antibody response by 2 weeks in those who are successfully colonized but not in those who are not colonized, implying that sustained exposure to an organism is required for optimal immune responses at this site [29]. The inoculum used in that study was low—$1 \times 10^4$ colony-forming units. In the present study we sought to expose human volunteers to a nasopharyngeal challenge cognate to their MCC vaccination. A safe alternative to polysaccharide inoculation might have been inoculation of killed serogroup C–expressing organisms, but the purified polysaccharide had the advantage of being easily titrated and of standard pharmaceutical grade. We chose a dose of $50 \mu$g as this would easily exceed the antigenic exposure caused by natural colonization by serogroup C–expressing organisms and likely overcome the lack of sustained stimulus. In the event we found that inoculation of this dose of polysaccharide was indeed immunogenic, but we assume that the scale and kinetics of induction of antibody we

**Figure 1.** MenC SBA kinetics in response to polysaccharide nasal rechallenge. One year postvaccination, subjects were challenged with nasal administration of 25 $\mu$g MenC polysaccharide vaccine, and SBA titers measured at 0, 4, 7, and 10 days post-rechallenge: (A) subjects with initial SBA titers $< 8$ ($n = 11$); (B) subjects with initial SBA titers $< 128$ ($n = 7$); (C) subjects with initial SBA titers $\geq 128$ ($n = 67$). GMT ±95% Confidence Interval. **P < .01, Friedman test with Dunn selected-pairs posttest (0 vs 4, 0 vs 7, and 0 vs 10).

**Table 4.** MenC Salivary IgA GMCs (ng MenC-Specific/μg Total IgA; 95% CI) 1 Year Postvaccination, Response to Polysaccharide Challenge, and Correlation With SBA Titers and IgG Concentrations

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<thead>
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<th>Parameter</th>
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<tbody>
<tr>
<td>No. of subjects</td>
<td>86</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>IgA GMC (95% CI)</td>
<td>10.4 (7.58–14.26)</td>
<td>7.8 (5.71–10.85)</td>
<td></td>
</tr>
<tr>
<td>Spearman $r$ (95% CI) with SBA titers</td>
<td>.14 (−.07–.35)</td>
<td>.14 (−.08–.34)</td>
<td></td>
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<tr>
<td>Spearman $r$ (95% CI) with serum IgG</td>
<td>.21 (−.002–.41)</td>
<td>.19 (−.02–.39)</td>
<td></td>
</tr>
<tr>
<td>Spearman $r$ (95% CI) with salivary IgG</td>
<td>.16 (−.05–.36)</td>
<td>.19 (−.02–.39)</td>
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The kinetics of rechallenge with MenC polysaccharide is depicted in Figure 1.
observed would be similar in those encountering bacteria naturally.

This study was performed using monovalent MenC conjugate vaccine, so although it is likely, we cannot be certain that these findings also apply to other non-B serogroups, such as serogroups A, W-135, and Y, which are included in new-generation quadrivalent conjugate vaccines.

In conclusion, we have found that there is loss of protective SBA levels in ~12% of young adults exactly 1 year post-vaccination with MCC. This group is able to mount a systemic, but not mucosal, immune response following nasal rechallenge with polysaccharide, demonstrating that immunological memory is intact; but the speed of this response may be too slow to provide protection from meningococcal infection, suggesting that persistence of bactericidal antibody may be more important in glycoconjugate vaccine–induced protection than immunological memory.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are posted to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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Potential conflicts of interest. A. W. H. has received grant support through his institution from Adjuvantix Ltd, Gates Foundation, and the National Institute of Allergy and Infectious Diseases and also holds stock options in Adjuvantix Ltd. R. C. R. has received grant support through his institution from Novartis Vaccines and Diagnostics. All other authors: no conflicts.

Table 5. MenC Salivary IgG GMCs (ng MenC-Specific/μg Total IgG; 95% CI) 1 Year Postvaccination, Response to Polysaccharide Challenge, and Correlation With SBA Titers

<table>
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<th>Parameter</th>
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<tr>
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<td>85</td>
<td>83</td>
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</tr>
<tr>
<td>IgG GMC (95% CI)</td>
<td>20.58 (12.81–33.08)</td>
<td>27.98 (18.12–43.22)</td>
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<tr>
<td>Spearman r (95% CI)</td>
<td>.51 (.33–.65)</td>
<td>.49 (.30–.64)</td>
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<tr>
<td>with SBA titers</td>
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<tr>
<td>Spearman r (95% CI)</td>
<td>.73 (.60–.81)</td>
<td>.74 (.61–.82)</td>
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<td>with serum IgG</td>
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References