Serum Antibody Kinetics following Nasal or Parenteral Challenge with Meningococcal Polysaccharide in Healthy Adults

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Limited data are available on the kinetics of meningococcal serogroup C (MenC)-specific antibody responses following parenteral or nasal challenge in those who have received prior MenC vaccination (polysaccharide or conjugate). Young adults who had previously received either meningococcal A/C polysaccharide (MACP) or MenC conjugate (MCC) vaccine or naive subjects were challenged with MACP via one of two routes, nasal or parenteral. Blood samples were taken prevaccination and on days 1 to 4 and day 10 postvaccination. MenC serum bactericidal antibody (SBA) and MenC-specific IgG were measured. Following parenteral challenge, MenC SBA and IgG responses were seen to occur between 4 and 7 days postchallenge. A lower proportion of subjects responded following nasal challenge, with naive subjects showing little change in SBA geometric mean titer (GMT) and IgG geometric mean concentration (GMC) over the 10 days following challenge. Increases in SBA GMTs were seen between 4 and 7 days after nasal challenge in those who had received prior MCC and between 7 and 10 days in those who had received prior MACP, and the responses in the prior-MACP group were of lower magnitude than the responses of the prior-MCC group. The data presented here indicate that, following MCC vaccination, memory has been induced at the mucosal level, and these subjects were able to respond with increases in SBA levels. These results demonstrate that the speed of response (primary or secondary) to challenge with MenC polysaccharide via the nasal or parenteral route does not differ and support concerns that immunological memory alone is too slow to provide protection.

The introduction of meningococcal serogroup C conjugate (MCC) vaccines into the routine immunization schedule in the United Kingdom, along with a catch-up campaign, significantly reduced reported cases of meningococcal serogroup C (MenC) disease (13). The induction of immune memory has been demonstrated following priming with MCC vaccines (4, 17, 18), but reports of secondary vaccine failures occurring 1 to 4 years after infant priming raises the question of whether anamnestic responses are sufficient for protection (2) and support concerns that encapsulated bacteria, such as meningococci, with the ability to rapidly invade the host may challenge the rapidity with which the immune system can generate anamnestic responses. Anamnestic responses require the reactivation of memory B cells and then their differentiation into antibody-producing cells.

The human nasopharyngeal mucosa is the natural reservoir of Neisseria meningitidis and, thus, is presumed to be the main site from which invasion into the bloodstream occurs. To be effective against colonization, vaccines must induce local immune responses which eliminate the pathogen. In young adults, both MenC polysaccharide and MCC vaccines have been shown to induce a significant production of mucosal antibodies in saliva (23), but mucosal MenC antibodies have been found to decline rapidly to near-prevaccination levels after 6 to 12 months (22). Therefore, the protection provided by these antibodies may be short term unless mucosal immunological memory is induced. Within the United Kingdom, the population impact on MenC disease has been maintained because of the striking reduction in MenC carriage as a result of vaccination, leading to herd immunity (10, 11, 16).

Knowledge of the pattern of antibody responses to polysaccharide challenge, in particular the immediate kinetics, in recipients primed with MCC vaccines is important in determining the response time from vaccination to the development of a protective antibody titer. Several studies have assessed the early kinetics of antibody responses to meningococcal vaccination, and after parenteral challenge with meningococcal polysaccharide, serum bactericidal antibody (SBA) responses were detected at day 5 in adolescents (19) and between 2 and 4 days in toddlers. However, in the latter study, different subjects were sampled at each visit (20).

The speed of the response to challenge may vary depending upon the route of challenge (nasal versus parenteral) and is likely to be different in naive subjects than in those primed with MCC vaccine or a polysaccharide vaccine. We report here on
the serum response to either parenteral or nasal administration of MenC polysaccharide in those who have previously received MCC or meningococcal serogroup A/C polysaccharide (MACP) vaccine and in meningococcal-vaccine-naïve subjects.

MATERIALS AND METHODS

Participants and recruitment. This study was conducted in February and March 2002, and subjects were recruited from The University of Sheffield Medical School. The meningococcal vaccination histories (dates and number of doses) of subjects were obtained from University Health records. Exclusion criteria (as per 1996 Immunization against Infection Disease issued by the Department of Health) were acute febrile illness on the day of vaccination, severe reaction to a previous dose of meningococcal vaccine, and known pregnancy. The study was approved by the Public Health Laboratory Service and South Sheffield local research ethics committees. Written informed consent was obtained from all subjects before enrolment.

Enrolment into the study, subjects were assigned to a group according to their vaccination history, as follows: (i) those naïve to any meningococcal vaccine, (ii) those who had received the serogroup C conjugate (MCC) vaccine, and (iii) those who had previously received MACP vaccine but had never received MCC vaccine. Subjects within each group were randomized to receive a challenge dose of MACP either nasally or parenterally.

Polysaccharide vaccination. The MACP vaccine used was Meningvac (A + C) (Aventis Pasteur, France), which contains 50 μg each of meningococcal serogroup A and C polysaccharide.

Nasal vaccination. For nasal vaccination, a full dose of MACP vaccine was dropped from a microsyringe into one nostril of a seated individual whose neck was fully extended. Each individual made an inspiratory sniff and then remained for 10 s following the procedure.

Parenteral vaccination. For parenteral vaccination, one-fifth of a dose of MACP vaccine (0.1 ml containing 10 μg of meningococcal serogroup C polysaccharide) was given by intramuscular injection into the deltoid of the non-dominant arm with a 1-inch, 25-gauge needle.

Following either route of vaccine administration, individuals remained under observation for 30 min.

Clinical samples. Clotted blood samples (5 ml) were taken immediately prior to MACP vaccine administration and then daily for 4 days and at days 7 and 10 postvaccination. Sera were separated and stored at −80°C for serological analysis.

Evaluation of immunogenicity. Functional antibody was determined by the MenC serum bactericidal antibody (SBA) assay using a standardized assay as previously described (12). The SBA target strain was C11 (C:16:P1.7-1,1), and the complement source was 4- to 6-week-old baby rabbit serum (Pelfreez, WI), beginning at a starting dilution of 4. SBA titers were expressed as the reciprocal of the final serum dilution giving ≥50% killing at 60 min. MenC-specific IgG was quantified using a standardized enzyme-linked immunosorbent assay (ELISA) (5), using CDC 1992 as the standard (8a). Antibody titers/concentrations below the limit of detection were arbitrarily assigned half the lower limit of detection; these were 2 and 0.00 μg/ml for the SBA assay and MenC-specific IgG, respectively.

Statistical analysis. An individual response was defined as a ≥4-fold increase in SBA titer or a ≥1.5-fold increase in MenC-specific IgG from day 0 (3). For each sampling time, the cumulative proportion of subjects who had a positive response by that time (i.e., there was an ≥4-fold SBA or an ≥1.5-fold IgG increase from day 0 on that day or any preceding day) was calculated. The proportion of responders, and the 95% confidence interval (CI), at each sampling point following MACP administration was calculated. Significant differences between the proportions of responders in the six groups at each sampling point were calculated using the Fisher exact test.

The antibody levels were log-transformed, and the geometric mean concentrations (GMCs) for IgG levels and SBA geometric mean titers (GMTs) were calculated, with the 95% CIs. Fold differences (with 95% CIs) in the GMT and GMC values of the groups at day 10 were calculated for each vaccine history group. The statistical significance of differences between the fold changes from baseline to day 10 in the groups were determined using a t test.

RESULTS

Study population. A total of 110 individuals were enrolled in the study; demographic and vaccine history details are shown in Table 1. Of the 56 subjects randomized into the group to be administered MACP nasally, 19 were naïve to any meningococcal vaccine, 19 had previously received MCC, and 18 had previously had MACP but not MCC. Of the 54 subjects randomized into the group to receive MACP parenterally, 19 were naïve to any meningococcal vaccine, 16 had previously received MCC, and 19 had previously had MACP but not MCC. The median times since previous meningococcal vaccination were similar between the parenteral and nasal groups for both the prior-MCC and prior-MACP subjects (Table 1). No date of prior meningococcal vaccination was provided for four subjects; three subjects had no date of previous MCC (two subjects were randomized into the parenteral group and one into the nasal group), and one subject had no date of prior MACP (randomized into the parenteral group).

Kinetics at an individual level. The proportion of responders at each sampling point was calculated, with the 95% CI. These data are presented in Tables 2 and 3. For the three groups who received parenteral MACP, individuals who responded with a ≥4-fold increase in SBA titer did so between days 4 and 7. The highest proportions of SBA responders were observed in the prior-MCC and naïve groups, with 66.7% (95% CI, 38.4, 88.2) and 77.8% (95% CI, 50.4, 93.6) of subjects, respectively, showing a response by day 7. This is compared to 26.3% (95% CI, 9.2, 51.2) of responders in the prior-MACP group. The MenC-specific IgG responses in those who received parenteral MACP mirrored the SBA responses, with those who responded doing so between days 4 and 7 and higher proportions responding by day 7 in the prior-MCC (80% [95% CI, 51.9, 95.7]) and naïve (66.7% [95% CI, 41.0, 86.7]) groups than in the prior-MACP group (31.6% [95% CI 12.6, 56.6]).

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### TABLE 1. Demographic characteristics of enrolled participants by group

| Characteristic | Prior MCC | Prior MACP | Naive |
|---------------|-----------|------------|-------|
| No. of subjects | 16 | 19 | 19 |
| % who were male | 56 | 42 | 37 |
| Mean age (yr ± SD) | 21.6 ± 2.2 | 20.6 ± 1.3 | 22.4 ± 1.2 |
| Median time [mo (interquartile range)] since prior vaccine | 26.5 (20.8, 27.0) | 27* (17.3, 27.0) | 30* (30.0, 45.3) |
| No prior MCC date is available for one subject. | No prior MCC date is available for one subject. | No prior MACP date is available for one subject. | NA |

* No prior MCC date is available for two subjects. 
* No prior MCC date is available for one subject. 
* NA, not applicable.
Compared to the responses to parenteral challenge, a lower proportion of subjects had SBA responses following nasal challenge. By day 4, there was a significant difference between the proportions of subjects with an SBA response in the six groups (P = 0.017), which was also true at days 7 and 10 (P < 0.001). Of those vaccinated parenterally who had received prior MCC, 33.3% (95% CI, 13.3, 53.9) of subjects demonstrated an SBA response at day 7, compared to 6.7% (95% CI, 0.2, 32.0) and 11.8% (95% CI, 1.5, 36.4) in the prior-MACP and naïve groups, respectively, whereas for MenC-specific IgG, a significant difference between the proportions of responders in the six groups was not seen until day 7 (P = 0.004).

Individuals who responded following nasal MACP administration did so between days 4 and 7. A similar proportion of subjects in the three groups showed a MenC-specific IgG response at day 7.

Kinetics at a population level. The MenC-specific IgG GMCs and MenC GMTs and 95% CIs by day for each of the six groups are shown in Tables 4 and 5, respectively. The SBA GMTs and MenC GMTs and 95% CIs by day for each of the six groups are shown in Tables 4 and 5, respectively. The SBA GMTs were also higher among individuals who received parenteral vaccination. This difference was statistically significant for those who had received previous MACP (P = 0.011), with a 3.03-fold (95% CI, 1.31, 7.04) difference between the two groups. The difference was also statistically significant for naïve subjects (P = 0.001), for whom the fold difference was greater, with an 8.12-fold (95% CI, 2.67, 24.68) difference between the two groups. As for MenC-specific IgG, the SBA GMTs were also higher among individuals who received parenteral MACP, but this difference was only statistically significant in naïve subjects (P < 0.001), with a 28.74-fold (95% CI, 6.33, 130.51) difference in the GMTs of parenteral and nasal groups.

Of those who received MACP nasally, the largest SBA GMT fold increase was observed in the prior-MCC group, with a 3.59-fold (95% CI, 1.50, 8.57) increase compared to fold increases of 1.24 (95% CI, 0.56, 2.75) and 1.59 (95% CI, 0.98, 2.56) in the prior-MACP and naïve subjects, respectively. For those vaccinated parenterally, the same pattern was observed for the MenC-specific IgG GMC fold changes from day 0 to day 10, with the largest fold increase, 13.11 (95% CI, 6.04, 28.45), observed in the naïve subjects, compared to fold increases of 3.53 (95% CI, 1.99, 6.26) and 2.36 (95% CI, 0.97, 5.75) in the prior-MCC and prior-MACP group, respectively. In those who received nasal MACP, the fold increases were similar for all vaccination histories, with fold increases of 1.35 (95% CI, 1.04, 1.75), 1.64 (95% CI, 1.16, 2.32), and 1.68 (95% CI, 0.9, 3.15) in the prior-MCC, prior-MACP, and naïve groups, respectively.

Comparison of MenC-specific IgG GMCs depending upon the route of MACP administration and on vaccination history showed the IgG GMCs to be higher among individuals who received parenteral vaccination. This difference was statistically significant for those who had received previous MACP (P = 0.011), with a 3.03-fold (95% CI, 1.31, 7.04) difference between the two groups. The difference was also statistically significant for naïve subjects (P = 0.001), for whom the fold difference was greater, with an 8.12-fold (95% CI, 2.67, 24.68) difference between the two groups. As for MenC-specific IgG, the SBA GMTs were also higher among individuals who received parenteral MACP, but this difference was only statistically significant in naïve subjects (P < 0.001), with a 28.74-fold (95% CI, 6.33, 130.51) difference in the GMTs of parenteral and nasal groups.
DISCUSSION

This is the first report, to our knowledge, to compare the antibody kinetics of parenteral or nasal challenge of MenC polysaccharide in naïve subjects and in those who have previously been vaccinated with MenC-containing polysaccharide or conjugate vaccines. MenC SBA and IgG responses were seen to occur between days 4 and 7, regardless of the challenge route.

The mechanisms of protection against MenC meningococcal disease have not been fully defined but are thought to rely on a combination of pre-existing SBA that may immediately neutralize invading bacteria at the time of exposure and reactivation of polysaccharide-specific memory B cells that are induced upon antigen exposure (15). Baseline SBA GMTs and IgG GMCs were higher in those groups which had received prior MenC vaccination, with approximately a third of subjects in each group with SBA titers of ≥8 (data not shown), a level considered to be protective (1). Following MenC vaccination, SBA titers peak 2 to 4 weeks following vaccination and have been shown to persist at this level in those vaccinated with MCC (6), but they decline to 5% of their peak value in those vaccinated with polysaccharide and remain above preimmunization levels for 10 years (21). However, in this study, there were no differences observed in the SBA titers approximately 2 years following polysaccharide or MCC vaccination.

The kinetics of the immune response to antigenic challenge via two routes, parenteral and nasal, were assessed to understand whether the speed or magnitude of the responses differed. Previous studies on antibody kinetics have presumed that challenge with an intramuscular dose of meningococcal polysaccharide mimics the antigenic exposure experienced by the human immune system. The human nasopharynx is the only natural reservoir of N. meningitidis, and invasion is presumed to occur from this site; therefore, nasal administration of meningococcal polysaccharide was used here to mimic natural exposure to the polysaccharide. Following parenteral challenge, increases in the SBA GMTs and IgG GMCs were seen to occur between days 4 and 7, which is in agreement with previous data on the speed of immune responses following parenteral vaccination (3, 7, 19). A few individuals showed SBA and IgG responses before day 4, but the majority did so between days 4 and 7. It has been well documented that MCC vaccines induce immunological memory, and therefore, an anamnestic response upon encounter with the MenC polysaccharide is expected. Despite immunological memory in those primed with MCC, the speed of the primary and secondary responses was similar, which is in concordance with the results of others (7, 19).

Differences in the quality of the immune response in those vaccinated parenterally were evident in the differing ratios of SBA to IgG at day 10, with ratios of 11.1 in those who had received prior MACP, compared to 65.6 and 31.7 in those who had received prior MCC and naïve subjects, respectively. MenC-specific IgG responses were generally higher in prior-MACP recipients, suggesting that functional antibody responses are specifically impaired. Vaccination with repeated doses of polysaccharide can induce the phenomenon of hyporesponsiveness, which is characterized by a decreased immune response upon repeated doses compared to the response in those who have received a single dose. At days 7 and 10, evidence of hyporesponsiveness was seen, with lower SBA GMTs in those who had received prior MACP than in naïve individuals.

Overall, following nasal MACP challenge, a lower proportion of subjects responded in all three vaccine history groups. Naïve subjects showed little change in their SBA GMTs and IgG GMCs over the 10 days following nasal challenge with MACP. In those who had received prior MCC, increases in SBA GMTs were seen between days 4 and 7 following nasal challenge, whereas in those who had received prior MACP, increases were seen between days 7 and 10. Along with a later increase following nasal challenge, the fold changes from day 0 to day 10 were also of lower magnitudes in those who had previously received MACP, and only one subject was classified as a SBA responder. The IgG GMCs at day 7 were similar between the prior-MCC and prior-MACP groups, and similar proportions of subjects were classified as responders in the three vaccination history groups. The lack of response to MACP intranasally in naïve subjects is of interest because nasopharyngeal colonization after natural exposure to N. meningitidis is associated with induction of immunity (8). This implies that whole organisms are required for induction of immunity at the mucosal site, even among adults, who likely would have been exposed to circulating N. meningitidis prior to enrolment in this study.

Parenterally administered meningococcal capsular polysaccharide-based vaccines can induce mucosal immune responses (14, 22), and MCC vaccination may induce memory at the mucosal level (23). The data presented here indicate that, following MCC vaccination, memory has been induced at the mucosal level, and these subjects were able to respond with increases in SBA. In those who have previously been vaccinated with MCC, the serum response to nasal MACP chal-
T able 5. Serogroup C SBA GMT by day and group

| SBA GMT (95% CI) at indicated no. of days following vaccination | Vaccination administration history | Macp Parenteral | Macp Nasal | Naïve Parenteral | Naïve Nasal |
|---------------------------------------------------------------|----------------------------------|----------------|------------|----------------|------------|
| Baseline                                                      | 3.46 (1.8, 6.6)                  | 3.46 (1.8, 6.6) | 3.46 (1.8, 6.6) | 3.46 (1.8, 6.6) | 3.46 (1.8, 6.6) |
| 10 days                                                       | 10.38 (6.29, 17.29)              | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) |
| 7 days                                                        | 10.38 (6.29, 17.29)              | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) |
| 4 days                                                        | 10.38 (6.29, 17.29)              | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) |
| 2 days                                                        | 10.38 (6.29, 17.29)              | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) |
| 1 day                                                         | 10.38 (6.29, 17.29)              | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) |
| 0.5 day                                                       | 10.38 (6.29, 17.29)              | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) | 10.38 (6.29, 17.29) |

The views expressed in the publication are those of the authors and not necessarily those of the Department of Health.

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