Mucosal immune responses to meningococcal conjugate polysaccharide vaccines in infants

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Background. Serogroup C meningococcal conjugate polysaccharide vaccines have been reported to induce significant serum IgG antibodies and immunologic memory in infants. Because meningococcus is a mucosal pathogen colonizing the nasopharynx, local mucosal immune responses may play an important role in host defense against infection and carriage. We have investigated the mucosal IgA and IgG antibody responses to two meningococcal C conjugate vaccines in the saliva of healthy infants.

Methods. Specific salivary IgA and IgG antibodies to two meningococcal C polysaccharide conjugate vaccines (Menjugate from Chiron Corp., n = 46; and Meningitec from Wyeth Lederle, n = 54) were investigated by immunoassay in infants after parenteral vaccinations at the ages of 2, 3 and 4 months. Unstimulated saliva samples were collected immediately before the first immunization and 1 month after the third immunizations. Forty healthy infants receiving the same routine vaccines but no meningococcal C vaccine were recruited as controls.
Results. There were significant increases in meningococcal C polysaccharide-specific IgG antibody concentrations postvaccination compared with prevaccination concentrations in both vaccinated groups (both \( P < 0.001 \)), but no change in the control group. There were no significant increases in specific IgA postvaccination geometric mean concentrations in either the vaccine or the control groups. The number of IgA positives postvaccination increased slightly in the Wyeth vaccine group vs. controls (\( P < 0.05 \)).

Conclusions. Significant salivary IgG antibodies to meningococcal C polysaccharide were observed after parenteral immunization with two meningococcal C conjugate vaccines, whereas there was no significant increase in specific IgA antibody levels for these two vaccines.

INTRODUCTION

Meningococcus is the most common cause of bacterial meningitis and septicemia in children in the United Kingdom. The proportion of cases caused by serogroup C Neisseria meningitidis increased in the UK during the late 1990s. Children younger than 2 years have the highest attack rates.\(^1\) Polysaccharide (PS) vaccines have been used against several encapsulated bacterial infections including Haemophilus influenzae, Streptococcus pneumoniae and N. meningitidis. Anti-capsular PS antibodies are bactericidal and are associated with protection. Meningococcal polysaccharide vaccines to several serogroups including C have been available for many years and are proved to be protective in adults.\(^2\) However, like other PS antigens they are poorly immunogenic in young children. Hyporesponsiveness has also been reported after repeated doses of meningococcal C polysaccharide in children as well as in adults.\(^3\)–\(^5\) Serogroup C meningococcal conjugate polysaccharide vaccines have been developed and recently licensed in the UK. It has been reported that these vaccines induce significant serum IgG antibody responses and immunologic memory in infants.\(^6\)–\(^9\) Because meningococcus is a mucosal pathogen colonizing the nasopharynx, local mucosal immune responses could play an important role in host defense against infection and carriage. We and others have reported mucosal IgG and IgA responses to such vaccines in adults and older children.\(^10\), \(^11\) Such responses could reduce nasopharyngeal carriage of meningococci and thus induce herd immunity, as has been seen with protein-conjugated polysaccharide vaccines against Haemophilus influenzae type b (Hib), widely used since the early 1990s.\(^12\), \(^13\)

In this study we report mucosal IgA and IgG antibody responses to two meningococcal C conjugate vaccines in the saliva of healthy infants.

MATERIALS AND METHODS

Study subjects, vaccines and immunizations. Group 1 [vaccine: Chiron meningococcal C (MenC) conjugate vaccine]. Forty-six healthy infants (24 male) age 7 to 10 weeks were studied who were recruited to a larger safety study (which involved 2500 healthy infants to assess the incidence of rare adverse events after Chiron MenC vaccine).\(^14\) All subjects were immunized at 2, 3 and 4 months of age with Chiron MenC conjugate vaccine (Menjugate) injected intramuscularly into the anterolateral aspect of the right thigh. Each 0.5-ml dose of the vaccine contains 10 \( \mu \)g of MenC PS conjugated to diphtheria toxoid (CRM197) protein carrier (12.5 to 33.3 \( \mu \)g) and 1 mg of aluminum hydroxide (adjuvant) per dose. Subjects also received routine doses of diphtheria, tetanus and whole cell pertussis-Hib conjugate (Aventis Pasteur or Evans/SmithKline Beecham or Behring/Cynamid) and oral polio vaccine (SmithKline Beecham). At the same time 40 healthy infants (21 male) receiving the same routine vaccines but no MenC vaccine were recruited as controls.

Group 2 (vaccine: Wyeth Lederle MenC conjugate vaccine). Fifty-four healthy infants (29 male) ages 7 to 10 weeks were studied who were recruited to a multicenter double blind Phase 2 immunogenicity trial.\(^15\) All subjects were immunized at 2, 3 and 4 months with either a pilot lot or manufacturing lot (allocated randomly) of Wyeth Lederle meningococcal C conjugate vaccine (Meningitec) injected intramuscularly into the anterolateral aspect of the right thigh. The immunogenicity of the 2 lots was equivalent.\(^15\) Each 0.5-ml dose of the vaccine contains 10 \( \mu \)g of MenC oligosaccharide conjugated to diphtheria toxoid (CRM\(_{197} \)) protein carrier (15 \( \mu \)g) and 0.5 mg of aluminum phosphate (adjuvant) per dose. Subjects also received routine doses of diphtheria, tetanus and whole cell pertussis-Hib conjugate vaccine [either Trivax (Evans/Wellcome)] mixed with Hibex (polysorbyribitol phosphate-tetanus toxoid; SmithKline Beecham) or ACTHIB diphtheria, tetanus and pertussis vaccine, Aventis Pasteur (AP polysorbyribitol phosphate-tetanus toxoid)] and oral polio vaccine.

Saliva samples. Unstimulated saliva samples were collected immediately before the first immunization and 1 month (4 to 6 weeks) after the third immunizations using a sponge swab, transported in dry ice and stored at \(-80^\circ\)C until assay. The period of saliva storage was 3 to 6 calendar months.

Each study was approved by the South Sheffield local research ethics committee, and written informed consent was obtained from all parents before enrollment.

Immunoassay for salivary anti-MenC PS-specific IgG and IgA antibodies. Specific salivary antibodies against serogroup C meningococcal polysac-
charides were determined with an enzyme-linked immunosorbent assay as described previously. In brief, immunoassay plates (Dynex, Chantilly, VA) were coated overnight at 4°C with meningococcal C PS (gift of Dr. George Carlone, CDC) 5 μg/ml diluted in phosphate-buffered saline (PBS), containing methylated human serum albumin 5 μg/ml (gift of Mike Bybel, Aventis Pasteur). After washes, 10% fetal bovine serum in PBS was added to block nonspecific binding. Diluted saliva samples (1/10 in PBS containing 10% fetal bovine serum) or standard serum (CDC 1992 reference, gift of Dr. George Carlone) were added to each well and incubated. Subsequently alkaline phosphatase-conjugated anti-human IgG (Sigma) was added for the IgG assay. Substrate (p-nitrophenyl phosphate) was added, and the plates were incubated further. Optical density (OD) at 405 nm was measured using a plate reader (Dynex), and concentrations of IgG were calculated by interpolation on the standard curve derived from serial dilutions of the reference serum (CDC 1992). For anti-meningococcal C PS-specific IgA measurements, the plates were incubated at room temperature (RT) on a horizontal rotator for 2 h after addition of samples. Murine monoclonal antibodies to human IgA (1/5000) was then added to the plates and incubated at RT for 2 h. Alkaline phosphatase-conjugated goat anti-mouse antibodies (Stratech, Luton, UK) were added, and the plates were incubated overnight at RT. Subsequent procedures were as for the IgG assay. We do not use IgG background plate because the background OD is generally very low. For anti-meningococcal C PS-specific IgA, a plate coated with PBS only was used to control background for each assay; the OD values of samples and references on the PBS plate were subtracted from those coated with meningococcal C PS antigen. The mean [95% confidence interval (CI) in parentheses] background OD values on the PBS plate for IgA were 0.069 (0.054, 0.079) for salivary samples and 0.064 (0.052, 0.077) for reference serum.

For each assay a sample was considered positive if the mean of the 3 sample ODs was higher than the reference serum. 0.079) for salivary samples and 0.064 (0.052, 0.077) for values on the PBS plate for IgA were 0.069 (0.054, 0.079) for salivary samples and 0.064 (0.052, 0.077) for reference serum.

For each assay a sample was considered positive if the mean of the 3 sample ODs was higher than the mean + 2 SD of the 10 preimmunization samples which were pretested by immunoassay with no detectable antibodies (OD values not significantly different from PBS background).

Statistical analysis. Antibody concentrations were logarithmically transformed, and geometric mean concentrations (GMC) with 95% CI were calculated for each study group. Antibody titers below the limit of detection were arbitrarily assigned to one-half the lower limit of detection for each assay. The lower limits of detection for MenC IgG and IgA were 8 and 4 ng/ml respectively. Comparisons between pre- and postvaccination GMC within groups were made with paired two-tailed Student t tests. The chi square test or Fisher’s exact test where appropriate was used to compare the proportion of positive samples between pre- and postvaccination. Statistical analysis was done using SPSS for Windows (Version 9.0, SPSS Inc., Chicago, IL). P < 0.05 was considered to be statistically significant.

RESULTS
Pre- and postvaccination meningococcal C PS-specific IgG and IgA salivary antibody concentrations and the number of antibody-positive samples are summarized in Table 1. There were significant increases in specific IgG antibody concentrations postvaccination compared with prevaccination concentrations in both vaccinated groups (both P < 0.001), but no change in the control group. There were no significant increases in specific IgA postvaccination geometric mean concentrations in either the vaccine or the control groups. The number of IgA positives postvaccination increased slightly in the Wyeth vaccine group vs. controls (P < 0.05; Table 1).

The specific IgG and IgA antibody concentrations did not differ between those receiving the pilot and manufacturing lots of the Wyeth vaccine (data not shown).

DISCUSSION
The development of MenC conjugate vaccines has used the same technology as the highly successful protein-polysaccharide Hib conjugate vaccines. Conjugation of the polysaccharide to a protein carrier switches the immune responses to the polysaccharide antigen from T cell-independent to T cell-dependent, which results in an effective primary immune response in all age groups, including infants. Hib infection is now rarely seen in the UK and its disappearance after the introduction of universal primary immunization in 1992 was more rapid than expected. This appears to have been a result of the induction of significant mucosal immunity and thus herd immunity through reduction in upper respiratory carriage rates. There is evidence that this mucosal immunity may have resulted from induction of significant antibody responses and that salivary IgG may be important as well as IgA.

It has been suggested that antibody responses to immunization with polysaccharide vaccines depend on a number of factors including age, vaccine dose and dose regimen and the degree of preexisting natural immunity. Different polysaccharide vaccines including meningococcal A and C and pneumococcal polysaccharide have resulted in dramatic reductions in disease incidence and, it is suggested, in herd immunity. Reduction in nasopharyngeal carriage after polysaccharide vaccination has also been reported. Mucosal IgA and IgG could be important in these effects, but the mechanisms by which these mucosal antibodies reduce carriage are
unclear. Both secretory IgA- and IgG-specific antibodies have been shown to inhibit Hib colonization. Although the capsule is not known as an adhesin, anti-capsular polysaccharide antibodies may inhibit the adherence by coating the surface of the bacteria and sterically hindering the adherence to the epithelium. Complement- and specific antibody-mediated phagocytosis could also be involved in the clearance of encapsulated bacteria from the mucosal surface.

In this infant study both Chiron and Wyeth MenC conjugate vaccines induced significant production of salivary MenC PS-specific IgG antibodies. For IgA-specific antibodies, although there was a relative increase in the percentage of positives in one vaccine group compared with the control group, the geometric mean concentrations of IgA were not significantly increased after three doses of either vaccine. Because these MenC conjugate vaccines induce strong serum IgG responses, the former finding is expected, because salivary IgG is likely to be derived from the serum pool. However, the latter is in contrast to our previous report of mucosal responses to a single dose of the same Chiron MenC conjugate vaccine in adolescents and also in contrast to a previous report in infants by Borrow et al., which showed significant salivary IgA production to a different vaccine. Salivary IgA is likely to be locally produced in secretory form. The demonstrated lack of significant local specific IgA production in infants (compared with adolescents) to the same Chiron MenC conjugate vaccine may be the result of a relative inefficiency of IgA induction mechanisms including T and B cell or antigen-presenting cell function in infants after parenteral immunization. The varying immunogenicities of different vaccine preparations might account for the apparent disagreement in the IgA results between this study and that by Borrow et al., in which a bivalent meningococcal A and MenC PS conjugate vaccine was used. The quantities of MenC PS and protein carriers were different (10 µg and 12.5 to 33.3 µg, respectively, in this study vs. 11.7 µg and 48.7 µg). Our preliminary results from another study involving a different MenC PS conjugate vaccine show salivary IgA as well as IgG production in infants after primary immunization (unpublished data). These results suggest that the induction of mucosal IgA immune responses in infants varies among different vaccine preparations. The relative inefficiency of significant production of salivary IgA after primary immunizations does not completely exclude the possibility of mucosal memory responses after reexposure to the antigen as we have previously shown for other polysaccharide antigens, although it should be noted that Nurkka et al. did not show evidence of mucosal memory in previously vaccinated Gambian children to

### Table 1: GMC of meningococcal C PS-specific IgG and IgA antibodies (ng/ml) and number of antibody-positive samples in saliva in infants immunized with Chiron and Wyeth MenC conjugate vaccines and in controls who did not receive MenC vaccination

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<td>GMC (ng/ml)</td>
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<td>GMC (ng/ml)</td>
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<td>5.06 (4.00, 6.17)</td>
<td>12.5 (12.5)</td>
<td>12.5 (12.5)</td>
<td>&lt;0.001</td>
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* Numbers in parentheses, 95% CI.
† Numbers in parentheses, percent.
NS, not significant.
vaccination with an meningococcal A/C conjugate vaccine.

The relative importance of mucosal IgA and IgG antibodies in modulating nasopharyngeal carriage of meningococcus remains uncertain. The immunologic functions of these mucosal antibodies are currently under study.

REFERENCES