

Mucosal Immune Responses to Meningococcal Group C Conjugate and Group A and C Polysaccharide Vaccines in Adolescents

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Previous studies in children have shown that *Haemophilus influenzae* type b (Hib) polysaccharide conjugate vaccines can reduce nasopharyngeal carriage of *H. influenzae* and provide herd immunity and suggest that this effect is mediated through mucosal antibodies. As this phenomenon may operate in other invasive bacterial infections which are propagated by nasopharyngeal carriage, mucosal antibody responses to meningococcal C conjugate and A/C polysaccharide vaccines were investigated. A total of 106 school children aged 11 to 17 years were randomized to receive a single dose of either conjugate or polysaccharide vaccine in an observer-blind study. Before and at 1, 6, and 12 months after immunization, samples of unstimulated saliva were collected and assayed by enzyme-linked immunosorbent assay for group C polysaccharide-specific immunoglobulin A (IgA), IgA1, IgA2 and secretory component, IgG antibodies, and total IgG and IgA. A subset of serum samples were also assayed for specific IgA and IgG antibodies. The concentrations of specific IgA and IgG in saliva were expressed both as nanograms per milliliter and as nanograms per microgram of total IgA or IgG. One month after immunization, significant increases in antibody titers (both IgA and IgG) were observed in saliva in both groups. There were significant subsequent falls in antibody titers by 6 months. Anti-meningococcal C-specific secretory component and IgA antibody titers were closely correlated ($r = 0.85$, $P < 0.001$), but there was no significant correlation between salivary and serum IgA titers, suggesting that IgA antibodies are locally produced. Significant correlation was found between salivary and serum IgG titers ($r = 0.52$, $P < 0.01$), suggesting that salivary IgG may be serum derived. Compared with polysaccharide vaccine, the conjugate vaccine induced significantly higher salivary IgG responses ($P < 0.05$), although there were no significant differences between salivary IgA responses to the two vaccines. The conjugate vaccine induced greater salivary IgG responses than a polysaccharide vaccine. Both vaccines induced significant salivary IgA antibodies. Further studies are needed to establish the functional significance of these mucosal responses.

The increased incidence of septicemia and meningitis due to group C *Neisseria meningitidis* in recent years in the United Kingdom, particularly among older children and young adults, has created considerable public concern (30). Currently licensed polysaccharide vaccines against group C meningococcal disease do not induce immunological memory and are poorly immunogenic in children under the age of 2 years (4, 13, 21). New conjugate vaccines have been shown to induce greater immunoglobulin G (IgG) and bactericidal antibody responses (S. Choo, Q. Zhang, J. Everard, C. Goilav, E. Hatzmann, J. Zuckermann, and A. Finn, Arch. Dis. Child **80**:A71, abstr. G207, 1999), to be immunogenic in infants, and to induce immunological memory (11, 22–24, 34).

Haemophilus influenzae type b (Hib) conjugate vaccines have been used successfully to prevent Hib-related invasive diseases (2, 3, 10) and have been shown to reduce nasopharyngeal carriage and to induce herd immunity (1, 27, 32, 33), while unconjugated polysaccharide (PS) vaccines have little effect on carriage (28). The reduction in Hib carriage suggests that the parenterally administered conjugate vaccine may induce significant local immunity and thus prevent colonization in the nasopharynx, while Kauppi et al. (17) have subsequently shown that salivary antibody responses are induced by administration

of the vaccine. Studies by the same group have also shown that intranasally administered anti-Hib capsular PS antibodies can prevent nasopharyngeal colonization with Hib in an infant rat model (18, 19). These results suggest that mucosal anti-PS antibodies may play a role in the eradication of nasopharyngeal carriage. Like Hib, *Neisseria meningitidis* resides in the mucosa of the nasopharynx, and mucosal immune responses may therefore play a significant role in host defense against the development of invasive meningococcal infection. It is also possible that meningococcal conjugate vaccines, like Hib conjugate vaccines, may induce specific local immune responses and reduce rates of nasopharyngeal carriage.

Little information is available about the mucosal immune responses induced by conjugate meningococcal vaccines and how they compare with those induced by PS vaccines. Although both IgA and IgG antibodies can be detected in mucosal secretions, the relative functional importance of these two isotypes in the context of mucosal infections is largely unknown. In this study, we describe mucosal IgG and IgA antibodies to group C meningococcal PS in the saliva of adolescents given either a conjugate vaccine or a PS vaccine parenterally. We also report the correlation between mucosal and systemic immune responses to the two vaccines.

MATERIALS AND METHODS

Study subjects and vaccines. A total of 106 healthy schoolchildren aged between 11 and 17 years were recruited in one center (Sheffield) as part of a randomized controlled two-center phase II immunogenicity study. Subjects were randomized to receive a single dose of either a meningococcal C conjugate

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vaccine (MC-conjugate) or a group A and C meningococcal PS vaccine (MACPS). This was an observer-blind trial, as the vaccines were not identical in appearance. Study participants were immunized by study nurses not otherwise involved in the trial in a separate area of the study clinic. In this way, the subjects were not aware of which vaccine they received, and the study observers remained blinded. (In total, 182 subjects were recruited to the study in the two centers. In addition to the data presented here, serological analysis for specific IgG by a modified enzyme-linked immunosorbent assay [ELISA] for detection of antibodies of higher avidity [14] and bactericidal antibodies was performed in the laboratories of Chiron Corporation, Emeryville, Ga., and is reported elsewhere [Choo et al., *abstr.*, 1999].) For the MC-conjugate vaccine, a 0.5-ml dose containing 10 µg of C PS conjugated to 10 µg of a nontoxic mutant of diphtheria toxin, CRM₁₉₇ (Chiron Vaccines SpA), was injected intramuscularly into the deltoid muscle. For the MACPS vaccine, a 0.5-ml dose of A/C PS vaccine [Meningivac (A+C); Pasteur Merieux, Lyon, France) containing 50 µg of each PS was also injected intramuscularly.

The study was approved by the South Sheffield local research ethics committee (protocol number 96/146), and written informed consent was obtained from all subjects and their parents before enrollment.

Sample collection. Before and at 1, 6, and 12 months after immunization, samples of unstimulated saliva were collected by soaking a sponge swab in the mouth until the swab was saturated with saliva. Samples were transported at 4°C to the laboratory within 3 h and stored at -70°C until assay. The period of saliva storage ranged between 1 and 6 calendar months. Venous blood samples were also collected, and the serum was separated within 3 h and stored at -40°C until analysis.

Immunoassay for salivary anti-meningococcal C PS-specific IgG, IgA, SC, IgA1, and IgA2 antibodies. Specific salivary antibodies against group C meningococcal PS were determined by a modification of the ELISA methods described by Kauppi et al. (17) and Carlone et al. (6). Immulon 1 microtiter plates (Dynex, Chantilly, Va.) were coated overnight at 4°C with meningococcal C PS (5 µg/ml; the gift of George Carlone, Centers for Disease Control [CDC]) diluted in phosphate-buffered saline (PBS; pH 7.3) containing methylated human serum albumin (5 µg/ml; George Carlone, CDC). After washes with washing buffer (PBS containing 0.1% Brij 35 [Sigma, St. Louis, Mo.]), 10% fetal bovine serum (FBS; Gibco, Paisley, United Kingdom) in PBS containing 0.1% Brij 35 was added and incubated at 37°C for 1 h to block nonspecific binding. Saliva samples were defrosted and immediately centrifuged at 10,000 × *g* for 10 min. Each sample supernatant was diluted 1:10 in PBS containing 10% FBS and added to the wells in triplicate. Serially diluted reference serum (CDC 1992 reference; the gift of George Carlone, CDC) was added at the same time to each plate. For the IgG assay, the plate was then incubated for 2 h at 37°C. After washes, alkaline phosphatase-conjugated anti-human IgG (Sigma) diluted 1:1,000 in PBS containing 10% FBS was added and incubated at 37°C for 2 h. After further washes, *p*-nitrophenyl phosphate (PNPP; Sigma) substrate (1 mg/ml) dissolved in diethanolamine buffer (pH 9.8) (Sigma) was added, and the plates were incubated for 1 h at 37°C. Optical density (OD) at 405 nm was measured with a plate reader (Anthos, Labtech International, East Sussex, United Kingdom), 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, secretory component (SC), IgA1, and IgA2 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 (1:5,000) (clone HP6123; Stratech, Bedford, United Kingdom), IgA1 (1:1,000) (clone A89-036; Nordic, Tilburg, The Netherlands), IgA2 (1:1,000) (clone A89-038; Nordic), or SC (1:5,000) (clone GA-1; Sigma), each diluted in PBS containing 10% FBS, were then added to the plates and incubated at RT for 2 h. Alkaline phosphatase-conjugated goat anti-mouse antibodies (Stratech) diluted 1:1,000 in PBS containing 10% FBS were added, and the plates were incubated overnight at RT. Subsequent procedures were as for the IgG assay. For anti-meningococcal C PS-specific IgA, SC, IgA1, and IgA2 measurements, 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 of samples run on the plate coated with meningococcal C PS antigen. The mean (95% confidence interval [CI]) background OD values on the PBS plate for IgA were 0.068 (0.054, 0.081) for saliva samples and 0.064 (0.052, 0.077) for the reference serum.

The CDC 1992 reference serum was used as a standard for both the IgG and IgA assays. This serum was also used as a reference standard for IgA1 by assigning it the arbitrary value of 1,000 U/ml. A saliva sample from an immunized subject containing a high titer of meningococcal C PS-specific IgA was used as a reference standard for anti-meningococcal C PS-specific SC and IgA2. Pooled serum from immunized volunteers was used as a quality control serum for the IgG and IgA immunoassays to control for day-to-day variations. The concentrations (nanograms per milliliter) of IgG and IgA anti-meningococcal C PS antibodies of unknown samples were calculated in each assay by interpolation on the standard curve derived from serial dilutions of the reference serum. Delta Soft software (BioMetallics Inc., Princeton, N.J.) was used to construct the standard curve, and a four-parameter curve-fitting method was used in the calculations. The relative concentrations of anti-meningococcal C PS-specific SC and IgA1 and IgA2 antibodies were expressed as units per milliliter, calculated from the reference curves in each assay as described above.

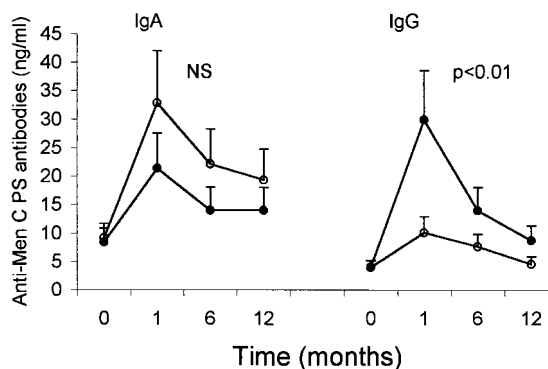


FIG. 1. GMC and 95% CIs of anti-meningococcal meningococcal C PS salivary IgA and IgG antibodies in adolescents before and after immunization with MC-conjugate vaccine (●) or MACPS vaccine (○).

Immunoassay for serum anti-meningococcal C PS IgG and IgA antibodies.

Serum anti-meningococcal C PS IgG and IgA antibodies were also measured by immunoassay. The method was as described above for the saliva assay except that the sample dilution used for the serum samples was 1:200 in PBS and all the incubations were done at RT.

Definition of antibody positivity. Ten preimmunization saliva samples with low levels (OD values not significantly different from background) of anti-meningococcal C PS specific antibodies were adsorbed with the PS antigen (20 µg/ml) for 1 h at RT before being tested by the immunoassay in triplicate. After adsorption and immunoassay for the 10 samples, the OD values changed little compared with non-adsorbed samples, suggesting that these samples contain little or no meningococcal C PS-specific antibody. The mean and standard deviation (SD) of the mean ODs of these 10 "negative" samples were then calculated. For each assay, a sample was considered positive if the mean of the three sample ODs was higher than the mean \pm 2 SDs of the 10 preimmunization samples. Serum anti-meningococcal C PS IgG and IgA positivity was also determined as above but instead using 10 preimmunization serum samples with low antibody levels.

Inhibition assay. To confirm the specificity of the salivary IgG and IgA immunoassays, inhibition assays were performed as described by Carlone et al. (6). Briefly, three postimmunization saliva samples diluted 1:10 in PBS buffer containing 10% FBS were used. Six 10-fold dilutions of meningococcal C PS in PBS buffer containing 10% FBS were prepared, starting at the highest concentration of 100 µg/ml. An equal volume of each PS dilution was added to aliquots of each of the diluted saliva samples in tubes, which were subsequently incubated on a horizontal rotator for 1 h at RT. The mixture was then added in duplicate to a microtiter plate, and a standard immunoassay procedure was followed. The percent inhibition for the three samples, for both IgA and IgG, when 100 µg of meningococcal C PS per ml was added, was between 78 and 95%. The concentrations of meningococcal C PS needed for 50% inhibition of antibody activity for the three samples, for both IgA and IgG, were within the range from 0.1 to 1 µg/ml.

Measurement of total salivary IgA and IgG. As salivary flow rates are known to vary between individuals and in the same individual under different conditions (9), total IgA and IgG were also measured, so that the ratio of anti-meningococcal C PS IgA or IgG to total IgA or IgG could be calculated for each individual subject as a method of compensating for dilution. Microtiter plates (Costar, Cambridge, Mass.) were coated with goat anti-human IgA or IgG (Dako, Glostrup, Denmark) in PBS (1:1,000 for IgG and 1:2,000 for IgA) and incubated overnight at 4°C. After washes (see the method described above), 10% FBS in PBS was added to each well to block nonspecific binding at RT for 1 h. Diluted saliva samples (1:200) along with the IgA and IgG standard (Sigma) were added in triplicate to the plates and incubated at RT for 2 h. Alkaline phosphatase-conjugated anti-human IgA or IgG (Sigma) diluted 1:2,000 in PBS was added after washes and incubated for 2 h at RT. After further washes, PNPP substrate was added, and the plates were incubated at RT for 15 min. ODs were read at 405 nm, and the concentration of each sample was calculated against the standard curve.

Statistical analysis. For the purpose of statistical analysis, all antibody concentrations were initially logarithmically transformed (base 10). Titers below the limit of detection were arbitrarily assigned to half the lower limit of detection for each assay. The lower limit of detection was determined from calculation of the lowest concentration in the standard curve derived from the reference standard using the Deltasoft software, the OD value of which was at least 2 SDs above the mean OD value of PBS blank controls (three plates loaded with PBS solution were assayed three times instead of test samples) for each assay. The lower limits of detection were 8 ng/ml for specific IgG, 6 ng/ml for specific IgA, 3 U/ml for specific SC and IgA1 and IgA2 antibodies, 1.3 µg/ml for total salivary IgG, and 0.6 µg/ml for total salivary IgA. When the specific salivary antibodies were

TABLE 1. Anti-meningococcal C PS-specific salivary IgA and IgG antibody concentrations in adolescents immunized with the MC-conjugate or MACPS vaccine

Day	GMC (95% CI), ng/ μ g			
	IgA		IgG	
	MC-conjugate	MACPS	MC-conjugate	MACPS
0	0.30 (0.24, 0.38) (<i>n</i> = 53)	0.30 (0.23, 0.39) (<i>n</i> = 53)	0.69 (0.56, 0.84) (<i>n</i> = 53)	0.71 (0.58, 0.87) (<i>n</i> = 53)
30	0.87 (0.63, 1.20) (<i>n</i> = 53)	1.15 (0.85, 1.54) (<i>n</i> = 53)	7.32 (4.71, 11.37) (<i>n</i> = 53)	2.66 (1.86, 3.80) (<i>n</i> = 53)
180	0.40 (0.31, 0.52) (<i>n</i> = 51)	0.53 (0.40, 0.70) (<i>n</i> = 52)	2.84 (1.74, 4.62) (<i>n</i> = 51)	1.60 (1.12, 2.27) (<i>n</i> = 52)
360	0.39 (0.30, 0.51) (<i>n</i> = 51)	0.48 (0.37, 0.63) (<i>n</i> = 51)	1.34 (0.90, 2.10) (<i>n</i> = 51)	0.76 (0.58, 0.98) (<i>n</i> = 51)

expressed as a ratio to total IgA or IgG, samples with undetectable specific IgA or IgG but detectable total IgA or IgG were assigned a value of 0.15 ng/ μ g for IgA and 0.5 ng/ μ g for IgG, which were the lowest values found, respectively. Geometric mean concentrations (GMCs) and 95% CIs were calculated by the study group for each time point. Two group comparisons were made using a two-tailed Student's *t* test. Serial measurements were analyzed using two summary measures: area under the curve (AUC) and the peak value (maximum concentration) for each subject during the time period of the study (25). The chi-squared test was used to compare positivity rates between groups. To assess correlations between two factors, Pearson's correlation coefficients were computed after log transformation and assessed for significance. Statistical analysis was done using SPSS for Windows (version 9.0; SPSS Inc., Chicago, Ill.). Values of *P* < 0.05 were considered statistically significant.

RESULTS

A total of 106 adolescents aged between 11 and 17 years entered the study, of whom 53 were randomized to the MC-conjugate group and 53 were randomized to the MACPS group. There were no significant differences between the two groups with respect to age [median [ranges], 13 years [11, 17] and 12 years [11, 17], respectively] and gender (male-female ratio, 27:26 and 26:27, respectively). Saliva samples were available for analysis from all 53 subjects in each group both before vaccination and 1 month (range, 1.0 to 2.0 months) after vaccination; from 51 in the conjugate and 52 in the PS group at 6 months (range, 4.0 to 8.2 months), and from 51 in each group at 12 months (range, 10.1 to 14.2 months).

Salivary anti-meningococcal C PS-specific IgA responses. Anti-meningococcal C PS-specific IgA responses are shown in Fig. 1 and Table 1. The GMC of IgA in both vaccine groups increased 1 month after immunization and fell subsequently over the next 6 to 12 months. There were no significant differ-

ences between the MC-conjugate vaccine and the MACPS groups' IgA responses when analyzed by summary measures either as peak values (*P* = 0.09) or as AUC (*P* = 0.08). The results were similar when analyzed using the ratio (nanograms per microgram) of the anti-meningococcal C PS-specific IgA to total IgA (Table 1) (*P* values, 0.24 and 0.18, respectively). The specific IgA positivity rates before vaccination were 42% (22 of 53) and 40% (21 of 53) for the conjugate and PS group, respectively (not significant [NS]). The equivalent data for the two groups 1 month after vaccination were 72% (40 of 53) and 85% (47 of 53) (NS).

To investigate whether the specific IgA detected was locally produced, we measured the anti-meningococcal C PS-specific SC (secretory IgA) concentrations in a randomly selected subgroup of subjects (28 in the MC-conjugate and 30 in the MACPS group) 1 month after immunization. There was a highly significant correlation between the anti-meningococcal C PS-specific IgA and the specific SC (secretory IgA) concentrations (*r* = 0.85) (Fig. 2). In contrast, there was no significant correlation between anti-meningococcal C PS-specific salivary IgA and the serum-specific IgA responses (*r* = 0.24; data not shown).

Anti-meningococcal C PS-specific IgA subclass (IgA1 and IgA2) concentrations were also measured in a randomly selected subgroup of subjects (24 per group) before and 1 month after immunization. Although the concentrations of both IgA1 and IgA2 subclass antibodies increased following immunization, there were no significant differences between the two vaccine groups in IgA1 and IgA2 concentrations or in the fold increase for the two subclasses (Table 2).

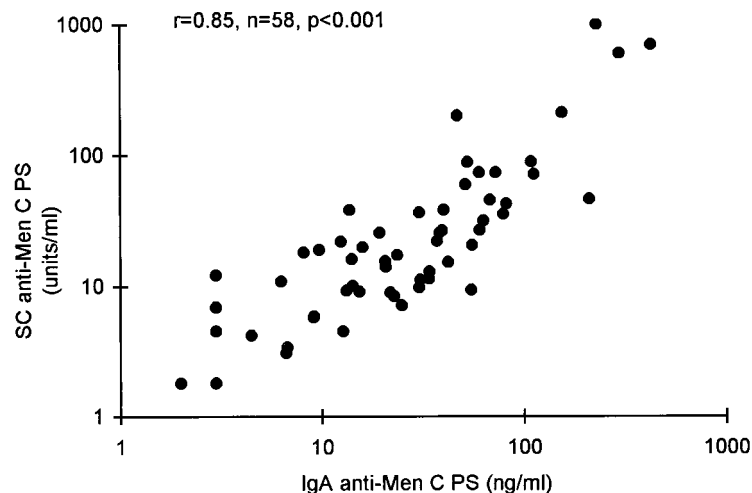


FIG. 2. Correlation between salivary anti-meningococcal C PS-specific SC and specific IgA antibodies 1 month after immunization.

TABLE 2. Anti-meningococcal C PS-specific salivary IgA1 and IgA2 antibody concentrations in adolescents before and 1 month after immunization with the conjugate or PS vaccine^a

Group	n	GMC (95% CI), U/ml			
		IgA1		IgA2	
		Before	After	Before	After
MC-conjugate	24	2.84 (1.81, 4.46)	4.81 (2.83, 7.62)	4.65 (2.83, 7.62)	14.06 (7.86, 25.12)
MACPS	24	2.33 (1.45, 3.75)	8.98 (5.01, 16.09)	3.81 (2.24, 6.48)	20.95 (8.90, 49.33)

^a Comparison between the two vaccine groups by a two-tailed Student's *t* test showed no significant differences.

Salivary anti-meningococcal C PS-specific IgG responses.

Anti-meningococcal C PS-specific IgG responses are shown in Fig. 1 and Table 1. Like IgA, salivary IgG concentrations rose at 1 month in both groups and then fell back towards baseline level at 6 to 12 months. The MC-conjugate vaccine induced significantly higher IgG response than the MACPS vaccine when summarized either as peak values ($P < 0.001$) or as AUC ($P < 0.01$). There was a significant correlation between salivary and serum IgG titers ($r = 0.52, P < 0.01$) (Fig. 3). The specific IgG positivity rates before vaccination were 13.2% (7 of 53) and 11.3% (6 of 53) for the conjugate and the PS group, respectively (NS); 1 month after vaccination, the specific IgG positivity rate for the conjugate vaccine group (79.2% [42 of 53]) was higher than that for the PS group (52.8% [28 of 53]) ($P < 0.01$).

Serum antibody responses. The MC-conjugate vaccine induced significantly higher serum IgG antibody levels than the MACPS vaccine (Table 3). However, there was no difference between the serum IgA responses to the two vaccines. Serum IgG positivity rates before vaccination were 18.2% (6 of 33) and 9.1% (3 of 33) for the conjugate and the PS group, respectively (NS). The equivalent data for the two groups 1 month after vaccination were 100% (33 of 33) and 90.9% (30 of 33) (NS). The equivalent data for serum IgA were 24.2% (8 of 33) and 21.2% (7 of 33), respectively, before vaccination (NS) and 97.0% (32 of 33) and 93.9% (31 of 33), respectively, after vaccination (NS) for the two groups.

DISCUSSION

The carriage of *N. meningitidis* in the nasopharyngeal mucosa in otherwise healthy humans has long been recognized. A

close relationship between the carriage rate in a population and the onset of an epidemic (12) suggests that treatment of carriage may decrease the incidence of disease. Hib conjugate vaccines have been shown to reduce nasopharyngeal carriage (27, 32, 33), and it has been suggested that local mucosal antibodies may be important in the reduction of carriage (17-19). It is plausible that newly developed meningococcal conjugate vaccines, which are similar in design to Hib conjugate vaccines, may induce effective local mucosal immunity against *N. meningitidis* in the nasopharynx. If such responses occur and if they are able to reduce the rate of asymptomatic carriage, this could greatly enhance the effectiveness of vaccines of this type when used widely, by the induction of herd immunity.

This study is among the first to examine mucosal immune responses to meningococcal vaccines. Specific mucosal immune responses of both the IgA and IgG isotypes were observed in saliva following parenteral administration of both the conjugate and the PS meningococcal vaccines. The conjugate vaccine induced significantly greater IgG antibody responses, but IgA responses were quantitatively similar for the two groups. This pattern was also seen in serum responses in this study. The relative size of the responses to the two vaccines should be interpreted in the light of different group C PS doses in the PS (50 µg) and conjugate (10 µg) vaccines. Nevertheless, our data suggest that the conjugate vaccine, which contains a protein carrier, causes immunoglobulin class switching more towards the IgG than the IgA isotype. Salivary antibody responses after a single dose of either vaccine subsequently fell back considerably towards baseline levels after 6 to 12 months.

Our observation that salivary and serum IgG concentrations are significantly correlated suggests that the specific IgG in

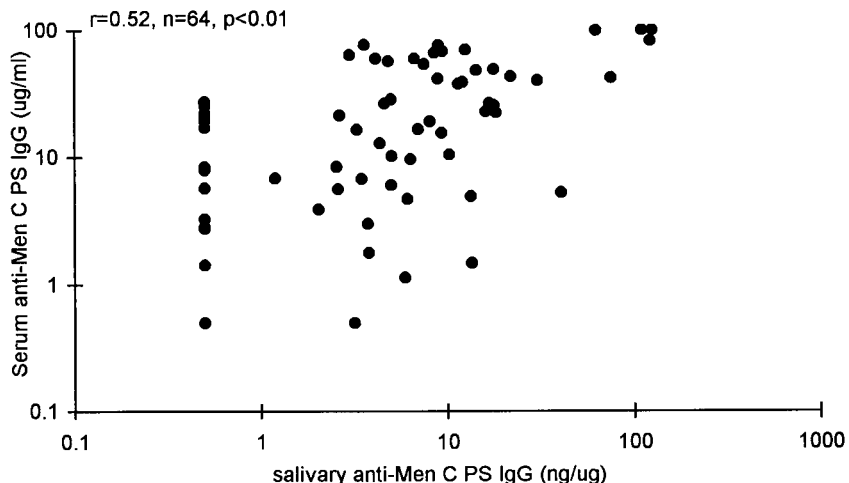


FIG. 3. Correlation between serum anti-meningococcal C PS-specific IgG and salivary specific IgG antibodies 1 month after immunization.

TABLE 3. Serum IgG and IgA antibodies to meningococcal C PS before (day 0) and 1 month after (day 30) immunization with MC-conjugate or MACPS vaccine

Group	n	GMC (95% CI), $\mu\text{g/ml}$			
		IgG		IgA	
		Day 0	Day 30	Day 0	Day 30
MC-conjugate	33	0.43 (0.279, 0.682)	30.07 (21.61, 41.83)	0.26 (0.176, 0.39)	3.49 (2.249, 5.429)
MACPS	33	0.39 (0.26, 0.58)	10.25 (6.44, 16.32)	0.27 (0.20, 0.36)	2.93 (2.00, 4.29)
<i>P</i> ^a		NS	<0.001	NS	NS

^a Comparison between the two vaccine groups by a two-tailed Student's *t* test.

saliva may be serum derived, as suggested previously with Hib vaccines (17). However, some IgG may be produced locally as well, as in some subjects the salivary and serum IgG antibodies were poorly correlated and the overall correlation coefficient was only 0.52. It is not known to what extent the rate of passive transudation of IgG from serum to mucosal surfaces varies between individuals or within individuals over time.

The close correlation between the anti-meningococcal C PS-specific SC (secretory IgA) and the specific IgA antibodies suggests that these IgA antibodies are secretory in form and thus locally produced. Poor correlation between salivary IgA and serum IgA antibodies suggests that the salivary IgA antibodies are less likely to be derived from serum than IgG and that their production may be regulated independently from serum responses following antigen stimulation. IgA exists as two subclasses, IgA1 and IgA2, which differ in their amino acid sequences and carbohydrate structures (15). The IgA1 protease produced by meningococcal strains may cleave IgA1, a mechanism which may facilitate bacterial colonization of mucosa by eliminating Fc-mediated functions of IgA1 antibodies (20). Due to the structural differences between IgA2 and IgA1, IgA2 is relatively resistant to this protease activity. This difference may provide some functional advantage for IgA2 in specific mucosal immune responses. As there are no standards for antimeningococcal-specific IgA1 and IgA2, we were unable to quantify IgA response in these two subclasses in absolute values. However, using reference sample standards, we show that both specific IgA1 and IgA2 levels were increased after immunization with both vaccines and that there were no significant differences between IgA subclass responses to the two vaccines. The GMC fold increase of IgA2 was slightly higher than that of IgA1 for both vaccines, but these differences were not significant.

The reported geometric mean quantities of specific IgG and IgA in the saliva of infants immunized with three doses of conjugate Hib vaccines, which are known to reduce nasopharyngeal carriage rates of that organism, vary between 14.4 and 31.0 ng/ml and between 20.0 and 43.6 ng/ml, respectively (17). This compares with similar values for salivary anti-meningococcal C PS antibodies observed after a single dose of the conjugate vaccine in adolescents in this study (IgG, 30.0 ng/ml; IgA, 21.4 ng/ml) (Fig. 1) and may suggest that this MC-conjugate vaccine could reduce nasopharyngeal carriage of this organism. The licensure and widespread use of conjugate meningococcal vaccines in the United Kingdom may provide an opportunity to directly observe effects on nasopharyngeal carriage rates.

It is not known how the intramuscularly injected vaccine antigens induce salivary responses. The induction and effector sites of these mucosal immune responses are unclear. It is possible that the PS antigen is absorbed into the body and

transported to lymphoid tissue local to the upper respiratory tract, as injected Hib PS antigen has been shown to be dispersed in the body (8, 31). Another possibility is that B cells are stimulated in the lymphoid tissues near the antigen injection site and then migrate to the upper respiratory mucosa and produce IgA antibodies there (5, 7, 26). It has been shown that antigen-specific IgA-secreting cells that bear the mucosal homing receptor $\alpha_4\beta_7$ can be detected in the circulation shortly after parenteral inoculation (16, 29), indicating that nonmucosal immunizations can induce effector B cells capable of homing to mucosal tissues. Further studies are needed to investigate these possibilities and the functions and mechanisms of action of these mucosal antibodies.

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