Primary and Booster Salivary Antibody Responses to a 7-Valent Pneumococcal Conjugate Vaccine in Infants

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Concise Communication

Salivary anticapsular antibody responses to a 7-valent pneumococcal conjugate vaccine (7VPnC) were measured in healthy infants. Infants received diphtheria-tetanus-pertussis/Haemophilus influenzae type b (DTP/Hib; group 1), DTP/Hib and 7VPnC (group 2), or DTP and 7VPnC/Hib (group 3) at ages 2, 3 and 4 months. All children received 23-valent pneumococcal polysaccharide vaccine at age 13 months. Salivary IgA and IgG responses to primary immunizations were generally poor. IgA mean concentrations at age 5 months were higher in the treatment groups than in control subjects for serotype 14 only ($P<.001$). At age 13–14 months, there were marked increases in IgA (mean fold difference, 3.7–4.9) and IgG (mean fold difference, 4.1–11.7) levels for serotypes 4, 9V, 14, and 19F and serotypes 4, 18C, 19F, and 23F, respectively, in the treatment groups. This contrasts with low IgA (1.2 and 1.4) and IgG (1.3 and 2.2) mean fold differences for non-7VPnC serotypes 1 and 5. The results suggest that 7VPnC primes for mucosal memory responses in infants.

Methods

Subjects. Healthy infants 6–10 weeks old were recruited from 1 UK center (Sheffield) as part of a 2-center randomized, controlled phase 2 immunogenicity trial. Serum data were collected from both sites, and salivary data were collected from Sheffield only.

Immunizations. Infants were randomized to 1 of 3 groups and were immunized at ages 2, 3, and 4 months. Group 1 received diphtheria-tetanus-whole cell pertussis vaccine (DTwP; Evans Medical, Leatherhead, UK) and Hib CRM197 conjugate vaccine (HbOC; Wyeth-Lederle) as a single injection. Group 2 received DTwP/HbOC and 7VPnC as 2 separate injections, and group 3 received HbOC/7VPnC and DTwP as 2 separate injections. Group 1 received 7VPnC at ages 5, 6, and 7 months. All infants received a PPS booster at age 13–16 months. 7VPnC contains 2 μg each of 4, 9V, 14, 18C, 19F, and 23F and 4 μg of 6B pneumococcal capsular saccharides conjugated to CRM197 protein. PPS contains 25 μg each of 6B pneumococcal capsular saccharides, including the 7VPnC serotypes and serotypes 1 and 5.

Saliva and blood samples. Saliva and blood samples were obtained before the first immunization, 3–9 weeks after the third immunization, before the booster immunization, and 3–9 weeks after the booster (ages 2, 5, 13, and 14 months). Unstimulated saliva samples were collected with a sponge swab, transported at 4°C, and stored at −70°C until assay. Saliva samples were analyzed in blocks (serotypes 4, 6B, 18C, and 23F; serotypes 9V, 14, and 19F) in order of subject number, because the saliva quantities obtained from most infants were too small to assay for all serotypes. Secretory component (SC) antibodies to serotype 14 were measured in the 14-month samples of a randomly selected subset of subjects from groups 2 and 3 ($n=28$). IgG and IgA anticapsular antibodies to serotypes 1 and 5 were measured in 13- and 14-month samples of another random subset of group 2 and group 3 infants ($n=68$). Salivary assays were performed at the University of Sheffield.
Salivary immunoassays. Salivary IgA and IgG antipneumococcal capsular polysaccharide antibodies against serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F and salivary SC anticapsular antibodies (secretory IgA) to pneumococcal serotype 14 were measured by ELISA, as described elsewhere [8], with the following modifications. Costar microtiter plates (Corning Inc., Corning, NY) were coated overnight at room temperature with each pneumococcal polysaccharide (American Type Culture Collection, Manassas, VA) at concentrations of 5–10 μg/mL in PBS. Saliva (1:10) and reference serum (1:50) samples were adsorbed with 15 μg/mL of pneumococcal C-polysaccharide in PBS containing 10% fetal bovine serum (Gibco, Paisley, UK) for 30 min at room temperature before they were added to the plate. Reference serum 89SF (from Carl Frasch, Food and Drug Administration, Bethesda, MD) with designated antibody levels was used as standard serum for the IgA and IgG assays. In the absence of a reference standard for specific anticapsular SC, optical density (OD) values were used to represent antibody levels for the SC assay.

Serum immunoassays. Serum IgG antibodies to 7VPnC type capsular polysaccharides were measured by standard ELISA methods [9].

Statistical analysis. IgA and IgG geometric mean concentrations (GMCs) at age 5 months were compared by using a 1-way analysis of variance model (3 levels) and an arbitrary significance level of .05, with vaccine group as the factor. Pairwise comparisons were made if significant differences were found (Bonferroni method). A paired sample t test (2-sided) was used to compare antibody concentrations at 13 and 14 months. Correlations between log-transformed data were tested, using Pearson’s correlation coefficient. Statistical analysis was performed with SPSS for Windows (version 9.0; SPSS, Chicago).

This study was designed to assess the serum immunogenicity of 7VPnC administered as a separate injection or as a combined HbOC/7VPnC injection at ages 2, 3, and 4 months [10]. As a secondary objective, salivary IgA and IgG responses to 7VPnC were assessed and are presented here.

Results

Paired saliva samples from 185 infants at primary phase (56, 66, and 63 in groups 1, 2, and 3, respectively) and 108 at booster phase (55 and 53 in groups 2 and 3) were available for analysis. Total salivary IgG and IgA antibody concentrations were measured and found to decrease between 2 and 5 months of age, increase at age 13 months, and increase further at age 14 months (data not shown). Antibody levels are shown in nanograms per milliliter and as a ratio of specific IgG or IgA to total IgG or IgA (ng/μL). Because conclusions following statistical analysis were similar, results are presented in nanograms per milliliter only.

Salivary IgA and IgG antibodies to 7VPnC. Salivary IgA GMCs at 5 months were significantly higher in both treatment groups than in the control group for serotype 14 only (P < .001; figure 1). IgA GMCs were also significantly higher in group 2 than in group 1 for serotypes 4 and 9V (P < .05) at 5 months. IgG responses at 5 months were even less impressive: GMCs were significantly higher in group 2 than in group 1 for serotype 4 only (P < .001; figure 2).

Salivary IgA GMCs were significantly higher at 14 months than at 13 months for all 7VPnC serotypes in group 2 (P < .05 for all) and for serotypes 4, 9V, 14, 18C, and 19F in group 3 (P < .01 for all; figure 1). In general, IgA mean fold differences at 13–14 months (1.3–5.8) for the treatment groups were higher than at 2–5 months (0.7–3.8). Marked increases in IgA levels at 14 months were apparent in group 2 for serotypes 4, 6B, 9V, 14, and 19F (mean fold difference, 4.1–5.8) and in group 3 for serotypes 4, 9V, 14, and 19F (mean fold difference, 3.7–4.1). Salivary IgG GMCs were significantly higher at 14 months than
Salivary anticapsular IgG antibody geometric mean concentrations (GMCs) and 95% confidence intervals at ages 2, 5, 13, and 14 months. Group 1: at ages 2 and 5 months; group 2: at ages 2 and 5 months, at ages 13 and 14 months; group 3: at ages 2 and 5 months, at ages 13 and 14 months; subset: at ages 13 and 14 months. 7VPnC, 7-valent pneumococcal conjugate vaccine; HbOC, *Haemophilus influenzae* type b CRM$_{197}$ conjugate vaccine.

at 13 months for all 7VPnC serotypes in group 2 (all $P < .05$) and for serotypes 4, 6B, 9V, 18C, 19F, and 23F in group 3 (all $P < .01$; figure 2). IgG mean fold differences for the treatment groups were generally higher at 13–14 months (1.2–11.7) than at 2–5 months (0.5–0.9). Marked increases in IgG concentrations at 14 months were observed in group 2 for serotypes 4, 9V, 18C, 19F, and 23F (mean fold difference, 4.6–10.5) and in group 3 for serotypes 4, 6B, 18C, 19F, and 23F (mean fold difference, 3.9–11.7).

**Salivary IgA and IgG antibodies to serotypes 1 and 5.** Salivary IgA GMCs at ages 13 and 14 months were not significantly different for non-7VPnC serotypes 1 and 5 (figure 1). IgA mean fold differences for these serotypes were very low (1.2–1.4). Salivary IgG GMCs at 13 and 14 months were not significantly different for serotype 1 and were significantly higher at 14 months than at 13 months ($P < .05$) for serotype 5 (figure 2). IgG mean fold differences for both serotypes were low (1.3–2.2).

**Nature of salivary IgG and IgA antibodies.** Salivary IgA antibody concentrations and salivary SC OD values for serotype 14 were correlated at 14 months ($r = 0.73$; $P < .001$). Salivary IgG antibody levels at 14 months correlated with serum IgG antibody levels for serotypes 4 ($r = 0.59$), 6B ($r = 0.53$), 14 ($r = 0.43$), and 23F ($r = 0.47$; all $P < .01$).

**Discussion**

To our knowledge, this study is the first to present evidence supporting an IgA mucosal memory response to pneumococcal conjugate vaccines in infants. These vaccines are thought to reduce nasopharyngeal carriage of vaccine-type pneumococci through the induction of local antipneumococcal capsular polysaccharide antibody responses. However, in our study, salivary IgA responses to 7VPnC after primary immunizations were generally poor, with significant responses to only 1 serotype (type 14). Specific salivary IgA antibodies were also detected rarely in Finnish infants immunized with 4-valent pneumococcal conjugate vaccines PncD or PncT at ages 2, 4, and 6 months [11]. At age 14 months, however, we observed substantial 4–5-fold increases in salivary IgA concentrations for serotypes 4, 9V, 14, and 19F in the treatment groups. This contrasts with very poor salivary IgA responses (1.2–1.4-fold increases) to serotypes 1 and 5 and suggests that 7VPnC primed for mucosal IgA memory responses for the above 7VPnC serotypes. Korkkeila et al. [11] also reported high IgA detection rates for serotypes 14 and 19F in PncD- or PncT-primed infants boosted with PPS at age 14 months. However, unprimed control subjects who received PncD at age 14 months had specific IgA detection rates similar to or higher than those of infants primed and boosted with PncD, which suggests that PncD did not prime for IgA memory responses in saliva.

In our study, specific salivary IgG concentrations decreased at age 5 months in both 7VPnC-primed and control infants. After booster, large 4–11-fold rises in IgG levels were observed in both treatment groups for serotypes 4, 18C, 19F, and 23F. This contrasts with mediocre 1.3–2.2-fold rises for serotypes 1 and 5, suggesting that IgG memory responses were detected in saliva for the above serotypes in 7VPnC-primed infants. Korkkeila et al. [11] reported low specific IgG detection rates at ages 7 and 15 months in PncD- and PncT-primed infants boosted with PncD or PPS. Serum IgG GMCs in Israeli and Finnish infants primed with 3 doses of PncD or PncT and boosted at ages 12–14 months with PPS were 0.9–5.9 µg/mL and 2.0–17.2 µg/mL, respectively, for the 4 vaccine serotypes [12–14]. In our study, serum IgG GMCs for these serotypes were generally higher (6.2–25.3 µg/mL at 14 months) [10], which may explain the higher salivary IgG concentrations in our study. Salivary
IgG correlated with serum IgG for most 7VPnC serotypes, suggesting that the salivary IgG was serum derived. Some salivary IgG may have also been locally produced, because correlation coefficients were not high. Salivary IgA correlated with salivary SC, suggesting that the salivary IgA was locally produced in secretory form.

Salivary antibody responses to pneumococcal polysaccharide vaccines in infants have not been previously investigated. Serum data suggest that both IgA and IgG responses to pneumococcal polysaccharide vaccines are generally poor in infants <2 years old, particularly with respect to serotypes 6B, 14, 19F, and 23F [15, 16]. In our study, salivary antibody responses to serotypes 1 and 5 were poor. Thus, although the dose of individual polysaccharides in PPS is higher than in 7VPnC, our results suggest that the mucosal immune system may have been successfully primed with 7VPnC for most 7VPnC serotypes. Some infants may also have been mucosally primed with wild-type pneumococci and/or cross-reacting antigens.

The mechanism by which parenteral administration of conjugate vaccines reduces nasopharyngeal colonization is not known. In an infant rat model, intranasally administered anti-Hib capsular polysaccharide IgG and IgA antibodies reduced Hib colonization, which suggests possible roles for both isotypes [17]. We observed poor salivary IgG and IgA responses to primary immunization with 7VPnC in our study. The quality of the mucosal antibody response, such as the degree of priming for mucosal memory, subclass distribution, avidity, and other functional aspects of these antibodies may, therefore, be important for the eradication of carriage in addition to antibody concentration.

In the United Kingdom, Hib vaccine boosters are not routinely given in the second year of life. If pneumococcal vaccine boosters are not given, the impact of pneumococcal conjugate vaccines on carriage could be reduced, resulting in less herd immunity. However, infants primed with pneumococcal conjugate vaccines may be sufficiently primed for mucosal memory responses after mucosal exposure to wild-type pneumococci, despite poor primary responses, and boosters may not be required. Thus, further studies are required to assess the need for boosters.

This study demonstrates the mucosal immunogenicity of a pneumococcal conjugate vaccine in British infants and provides evidence of priming for mucosal memory responses. More research is needed to investigate the function of salivary anti-capsular antibodies and the capacity of conjugate vaccines to induce immunologic memory at the mucosal level.

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