Pneumococcal Carriage and Otitis Media Induce Salivary Antibodies to Pneumococcal Surface Adhesin A, Pneumolysin, and Pneumococcal Surface Protein A in Children

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Local antibodies probably contribute to defense against Streptococcus pneumoniae. This study examined whether pneumococcal carriage and acute otitis media (AOM) induce mucosal antibodies to potential vaccine candidates pneumococcal surface adhesin A (PsaA), pneumolysin (Ply), and pneumococcal surface protein A (PspA). IgA to all 3 proteins was detected by EIA in saliva of 329 children at ages 6, 12, 18, and 24 months and of 17 adults. A higher proportion of IgA-positive samples and higher antibody concentrations were seen in children with pneumococci-positive cultures of nasopharyngeal samples or middle ear fluid than in children with all cultures negative for pneumococci. The strong correlation between IgA and the presence of the secretory component suggests that the IgA was secretory. The findings indicate that pneumococcal carriage and AOM induce local production of anti-PsaA, anti-Ply, and anti-PspA antibodies early in life.

Streptococcus pneumoniae (pneumococcus [Pnc]) is an important bacterial pathogen worldwide. It causes both invasive infections (e.g., pneumonia, meningitis, and septicemia) and local infections (e.g., sinusitis and acute otitis media [AOM]). Every year, ~3 million children die of pneumonia, most in developing countries. Pnc is the most important bacterial cause of pneumonia in young children and is responsible for a high proportion of these deaths [1]. In developed countries, AOM caused by Pnc is one of the most common infectious diseases in young children [2, 3]. Pnc is also a common component of the normal pharyngeal flora in healthy children and adults. The factors that lead to the development of Pnc carriage to Pnc disease are still largely unknown.

Pnc infection begins on the mucosal surfaces of the upper respiratory tract, and local immunity may have an important role in the defense against Pnc carriage and subsequent disease. The mucosal antibodies may inhibit the adherence of Pnc to its receptor molecules or may interfere with the replication of the bacteria on mucosal surfaces [4, 5]. By inducing mucosal antibodies, for example, by administration of the antigen locally by the intranasal or oral route the rate of Pnc carriage could potentially be reduced, and droplet spread of Pnc possibly could be diminished.

The currently available pneumococcal 23-valent polysaccharide (PS) vaccine poorly induces antibody responses in young children (<2 years old) and thus does not effectively protect against either invasive or local Pnc infections [6–8]. Covalent conjugation of PS antigens to a protein carrier improves their immunogenicity [9, 10], and such vaccines are highly protective in infants and children [11, 12]. They also can induce mucosal immune responses in infants and toddlers [13, 14]. The serotype selection of conjugate vaccines is more restricted than in PS vaccine, and the production costs are high. This will restrict the use of these vaccines, especially in economically poor developing countries.

The problems with the PS and conjugate vaccines have stimulated an interest in Pnc proteins and the possibility of developing vaccines that incorporate Pnc proteins. Three surface proteins, pneumococcal surface adhesin A (PsaA) [15–17], pneumococcal surface protein A (PspA) [18–20], and pneumococcal surface protein C (PspC) [21–23], and an intracellular toxin, pneumolysin (Ply) [24, 25], are now being considered as candidates for a Pnc protein vaccine. Each has induced protection in animal models [23, 26, 27]. These proteins offer several major advantages as a Pnc vaccine. First, as thymus-dependent antigens they are expected to be immunogenic, even in young children, and to induce
immunologic memory. Second, they would protect against Pnc regardless of serotype [26, 28], and third, they could be produced in large quantities at low cost with recombinant technology in an appropriate host, such as Escherichia coli [29–31] or Bacillus subtilis [32–34]. Thus, the Pnc proteins could fill the gaps in protection provided by the Pnc PS or conjugate vaccines and even act as stand-alone vaccines.

Because the natural development of local immunity against Pnc has not been extensively studied in young children, we performed a study to analyze how the concentration of IgA antibodies to the Pnc proteins in saliva develops by age and whether Pnc carriage and/or Pnc AOM is capable of inducing local production of IgA antibodies to these proteins. The IgA anti-PsaA, anti-Ply, and anti-PspA antibodies and the secretory component (SC) in anti-PsaA, anti-Ply, and anti-PspA were determined in saliva samples of children at ages 6, 12, 18, and 24 months and in adults. The serum IgG anti-PsaA, anti-Ply, and anti-PspA antibodies of the same children were assayed in a separate study [35, 36].

Materials and Methods

Study Cohort and Study Design

Study subjects were 329 children in the Finnish Otitis Media (FinOM) Cohort Study. They were enrolled on their second routine visit at the Hervanta Child Health Center in Tampere (Finland) at age 2 months and were followed up until their second birthday. A special study clinic with 2 doctors and 2 nurses was established in the Hervanta health center for this study from April 1994 to July 1997. The children were invited to the study clinic for scheduled visits 10 times between ages 2 and 24 months. During these “healthy visits” at ages 2, 3, 4, 5, 6, 9, 12, 15, 18, and 24 months, interview data and nasopharyngeal swabs (NPSs) were obtained. The saliva samples were collected at the 6-, 12-, 18-, and 24-month visits. If children developed respiratory illness or symptoms suggesting AOM, parents were asked to bring the child to the study clinic; this event was recorded as a “sick visit.” During the sick visit, the child’s history was obtained from the parent, a nasopharyngeal aspirate (NPA) was obtained, and the child had a physical examination by the study doctor, which included otologic examination with pneumatic otoscopy and tympanometry. Myringotomy with aspiration of middle ear fluid (MEF) was performed in cases of AOM diagnosed by pneumatic otoscopy, which suggested effusion in the middle ear cavity concomitantly with signs or symptoms of acute infection. We also obtained saliva samples from 17 healthy children (15 women and 2 men; mean age, 35 years; with and without a family and residence in Helsinki area), to measure the natural level of antibody concentrations to the 3 Pnc proteins in saliva of the adult population.

Clinical Samples

NPSs were obtained by study nurses through a nostril with a swab with a flexible aluminum wire shaft and calcium alginate tip (Calgiswab; Orion Diagnostica). NPA were obtained with a pediatric mucus extractor (Orion Diagnostica) by guiding the catheter through the nostril to the nasopharynx and by applying a gentle suction with an electric suction device. The study doctor performed myringotomy in all children with AOM. Unstimulated saliva samples were collected by placing a plastic pipette in the cheek area and by applying gentle suction. Saliva samples immediately were frozen and were stored at −70°C for further analysis. Samples were thawed only once and were centrifuged at 19,000 g for 10 min before assays. The supernatants were used for the measurement of antibodies.

Culture Methods

NPS, NPA, and MEF samples were plated immediately on chocolate agar and selective sheep blood agar (containing 5 μg of gentamicin) plates. These were incubated overnight at 36°C with 5% CO2 at the study clinic. The next day, plates were transported to the bacteriologic laboratory at the National Public Health Institute (Oulu, Finland), where they were further incubated for 24 h. Pnc was identified by standard methods [37].

Antigens

PsaA. The recombinant PsaA was prepared with the Qiagen system (Qiagen) by J. Sampson and E. Ades (Centers for Disease Control and Prevention [CDC], Atlanta). The expression host E. coli SG 13009 (pREP4) was transformed with pAB247, the recombinant plasmid that carries the psaA gene from the serotype 2 strain D39 cloned into pQE30. The His-tagged recombinant PsaA was purified by Ni-NTA chromatography [29].

Ply. The recombinant Ply used (J. Paton, Women’s and Childrens Hospital, Adelaide, Australia) was a derivative of Ply with a Trp433-Phe mutation, which reduces the hemolytic activity without affecting antigenicity. The antigen was purified from recombinant E. coli, as described elsewhere [30].

PspA. We used recombinant PspA representing the 315-aa N-terminal half of the Rx1 PspA produced in E. coli (R. Becker, Aventis, Swiftwater, PA). PspA is variable in structure, but different PspA share cross-protective epitopes. The 315-aa fragment of Rx1 is expected to contain sufficient epitopes for cross-reactivity of antibodies produced to different PspAs [31, 38, 39].

Serologic Assays

We used an EIA to determine the concentrations of IgA and SC for anti-PsaA, anti-Ply, and anti-PspA antibodies in saliva samples. The methods used for serum antibodies [35, 36] were modified for saliva samples on the basis of previous experience [40].

IgA-specific assay. Microtiter plates (Costar 3591) were coated with PsaA and PspA by incubating the plates overnight at 4°C and with Ply overnight at 37°C. The coating concentrations were as follows: 5 μg of PsaA and Ply and 150 ng of PspA in 1 mL of PBS. The plates coated with PBS only were used as blank plates for each sample for subtraction of nonspecific binding. The plates were washed between steps 4 times with PBS containing 0.05% Tween 20 (Tweens-PBS) by SkanWasher 300 (Skatron Instruments), except before the substrate (3 times with Tween-PBS and twice with distilled water). The plates were blocked with 10% fetal bovine serum (FBS; Gibco) in PBS (FBS-PBS) by incubation for 1 h at 37°C. FBS-PBS was used further as dilution buffer for saliva sam-
bles, monoclonal antibodies (MAbs), and polyclonal antibodies. The saliva samples were diluted 1:10 and were analyzed in triplicate. The diluted samples and reagents were pipetted (50 μL/well). MAbs to human immunoglobulins were used to determine IgA (M26012; Bionostics or Skibio). Incubation of samples and anti-human IgA MAbs was done on a horizontal rotator (200 rpm) for 2 h at room temperature. The polyclonal alkaline phosphatase-conjugated rabbit anti-mouse IgG antibodies (H&L 315-055-045; Jackson Immunoresearch Laboratories) were incubated without rotation overnight at 22°C. The substrate solution containing 1 mg of p-nitrophenyl phosphate disodium (Sigma104; Sigma Immuno Chemicals) in 1 mL of carbonate buffer (pH 9.8) was pipetted at 50 μL per well, and the plates were incubated for 1 h at 37°C. Optical densities (ODs) were measured at the 405-nm wavelength with an EIA reader (Titertek; Labsystems).

OD readings of each well on the PBS blank plate were subtracted from those on the PsaA, Ply, and PspA plates. The OD readings on the PBS blank plates were 0.05–0.70 (median, 0.085). An OD ≥0.05 for anti-PsaA, ≥0.04 for anti-Ply, and ≥0.03 for anti-PspA was considered to be positive (detection limit was calculated from OD readings given by blank wells on the PsaA, Ply, and PspA plates; 2 × SD of daily assays). Results were calculated as units of IgA per milliliter of saliva (U/mL) by a nonparametric fit, the 3-point cubic spline, which compared the mean OD readings of triplicate samples with a reference curve drawn on the basis of OD values of in-house reference serum (adult serum pool). The reference curve was considered to contain 100 U/mL of IgA anti-PsaA, anti-Ply, and anti-PspA antibodies. Samples with undetectable anti-PsaA, anti-Ply, and anti-PspA were assigned values of 0.1, 0.08, and 0.08 U/mL (respectively), which are equivalent to half the detection limits (0.2 U/mL for anti-PsaA, 0.16 U/mL for anti-Ply, and 0.16 U/mL for anti-PspA).

The day-to-day variation of the IgA-specific assay was followed with an adult saliva sample (1:10 dilution analyzed in triplicate) containing abundant IgA anti-PsaA, anti-Ply, and anti-PspA. This sample contained 24 U/mL of anti-PsaA, 26 U/mL of anti-Ply, and 17 U/mL of anti-PspA. The coefficients of variation (CVs) of different daily assays were 22, 19, and 27, respectively.

**SC-specific assay.** The concentration of the salivary SC in anti-PsaA, anti-Ply, and anti-PspA was measured in a subcohort of 168 children by ELISA, as described above, by using MAbs to human SC (I-6635; Sigma). An OD ≥0.03 for SC in anti-PsaA, anti-Ply, or anti-PspA was considered to be positive. Results are expressed as mean OD values, which were calculated from the OD readings of triplicate samples after subtraction of the OD readings of the PBS blanks. Samples with undetectable SC in anti-PsaA, anti-Ply, and anti-PspA were assigned a value of 0.015, which is equivalent to half the detection limit. The day-to-day variation of the SC-specific assay was followed with an adult saliva sample (1:50 dilution). ODs for anti-PsaA, anti-Ply, and anti-PspA were 0.9, 0.9, and 0.8, respectively. The CVs of different daily assays were 19, 19, and 28, respectively.

**Pneumococcal Culture Findings**

To evaluate the relationship between the Pnc-positive (Pnc+) culture findings and salivary antibody production, we grouped the children at each time point into 2 categories as follows: Pnc-negative (Pnc-) children with no Pnc+ NPS, NPA, or MEF cultures on healthy or sick visits up to the time point in question and Pnc+ children with ≥1 Pnc+ NPS, NPA, or MEF culture on healthy or sick visits up to the time point in question. The Pnc- children were subgrouped further in 2 categories: Pnc- carrier children with ≥1 Pnc+ NPS culture on healthy visits but no Pnc- NPA or MEF culture on sick visits up to the time point in question and Pnc- sick children with ≥1 Pnc- NPA or MEF culture on sick visits up to the time point in question (regardless of result of NPS cultures done during scheduled healthy visits). A child’s category could change from one time point to another from a Pnc- to a Pnc+ category or from the Pnc- carrier category to the Pnc+ sick category, but never vice versa. Pnc+ sick children were not further subgrouped into separate NPA-positive and MEF-positive categories, because no difference was detected at any time point between the salivary antibody concentrations in these 2 groups of children.

**Statistical Methods**

We used SPSS (SPSS) and Epi Info version 6 (World Health Organization) software for statistical comparisons that were performed on log-transformed data. We compared the concentrations of IgA anti-PsaA, anti-Ply, and anti-PspA in saliva of the Pnc- and Pnc+ children by Student’s t test. The IgA concentrations in the Pnc- category and in the 2 Pnc+ categories were compared by 1-way analysis of variance, followed by a post hoc test (Tukey honestly significant difference test) when appropriate. The proportions of IgA-positive samples in the Pnc- and Pnc+ children were compared by Yates’s corrected χ² test or Fisher’s 2-tailed exact test. We used Pearson’s correlation to evaluate the correlation between anti-PsaA, anti-Ply, and anti-PspA measurements and the IgA- and SC-specific assays.

We summarized the development of salivary IgA anti-PsaA, anti-Ply, and anti-PspA concentrations by age by box plots based on the median, quartile, and extreme values (i.e., cases with values >3 box lengths from the upper or lower edge of the box). A line across the box indicates the median.

**Results**

Of the 329 children enrolled in the FinOM Cohort Study, 281 completed follow-up at age 24 months. At age 6 months, 105 (33%) of 319 children were Pnc- (i.e., had ≥1 Pnc+ NPS, NPA, or MEF culture finding). By age 24 months, 244 (87%) of 281 children were Pnc+. Saliva samples were obtained from 317 children; 4 saliva samples from 211 children were obtained at ages 6, 12, 18, and 24 months; 3 samples were obtained from 68 children; 2 from 29; and 1 sample from 9 children.

**Development of IgA anti-PsaA, anti-Ply, and anti-PspA antibodies in saliva by age.** By age 6 months, anti-PsaA was found in the saliva of 171 (57%) of the 300 children and anti-Ply in the saliva of 255 (85%) of the 300 children, whereas anti-PspA was found in the saliva of only 25 (9%) of the 272 children
(table 1). By age 24 months, 193 (74%) of the 261 children had antibodies to PsaA and 242 (93%) of the 261 children to Ply but only 53 (23%) of the 235 children to PspA. In the saliva of adults, anti-PsaA and anti-Ply were detected in all samples and anti-PspA in 15 (88%) of 17.

The GMCs of the salivary IgA anti-PsaA, anti-Ply, and anti-PspA increased steadily with age (table 1). Despite the increase, the GMCs of anti-PsaA, anti-Ply, and anti-PspA did not reach the antibody concentrations of adults by age 24 months (0.62, 2.01, and 0.13 U/mL, respectively). Antibody concentration differences between adults and children were statistically significant at ages 6 and 12 months (0.31, 0.70, and 0.01 U/mL, respectively) by age 24 months (0.62, 2.01, and 0.13 U/mL, respectively). The GMCs of anti-PsaA, anti-Ply, and anti-PspA did not reach the GMCs of anti-PsaA, anti-Ply, and anti-PspA in adults (1.96, 6.11, and 0.79 U/mL, respectively). Despite the increase, the antibody levels remained lower than those in adults (figure 1). The variation in the antibody concentration among individuals was large in both children and adults. Most anti-PspA values in children were below the detection limit, and all positive samples shown are outliers.

**Correlation between IgA anti-PsaA, anti-Ply, and anti-PspA antibodies in saliva.** To test whether the IgA anti-PsaA, anti-Ply, and anti-PspA antibodies were produced simultaneously, we evaluated the correlation among all anti-PsaA, anti-Ply, and anti-PspA results separately in Pnc+ and Pnc− children. The correlation between anti-PsaA and anti-Ply was the strongest both in Pnc+ children (r = 4; P < .01) and Pnc− children (r = 3; P < .01). The correlation between anti-PsaA or anti-Ply with anti-PspA was poor, probably because of the few samples positive for anti-PspA; however, the correlation was significant in the Pnc+ children in both cases (r = .1; P < .01–.05).

In adults the correlations were not significant, which is probably because of the small number of samples.

**Nature of salivary IgA.** To determine whether the salivary IgA anti-PsaA, anti-PspA, and anti-Ply antibodies in children were locally produced, the OD values of IgA were compared with those of SC in anti-PsaA, anti-Ply, and anti-PspA in a subpopulation of 168 children (figure 2). We combined the OD values for IgA and SC measured at different time points, since the correlation at different ages was similar. The correlation of IgA with SC in the anti-PsaA, anti-Ply, and anti-PspA was highly significant (r = .94, r = .83, and r = .80, respectively; P < .01, for all comparisons). In all, 68 (14%) if 499 samples contained anti-PspA IgA, even at high concentrations, but did not contain SC (figure 2).

**Comparison of salivary IgA antibodies to Pnc culture findings.** The respective proportions of the IgA samples positive for anti-PsaA, anti-Ply, and anti-PspA at all time points were higher in Pnc+ than in Pnc− children (table 1). The proportion of the anti-PsaA−positive samples at all time points was ~1.5–2 times greater in Pnc+ than in Pnc− children (P < .001, all comparisons). The proportion of the anti-Ply−positive samples at age 6 months was about the same in Pnc+ and Pnc− children, but, at later ages, the proportion of positive samples was slightly higher in Pnc+ children (P < .01 at 18 months). The proportion of anti-PspA−positive samples increased steadily with age in Pnc+ children but remained the same or decreased in the Pnc− children. At age 6 months, the proportion of the anti-PspA−positive samples was 2 times greater in Pnc+ than in Pnc− children (P < .05); at age 24 months, there was an 8-fold difference between the categories (P < .001).

The respective GMCs of IgA anti-PsaA, anti-Ply, and anti-PspA antibodies at all time points were significantly higher in Pnc+ than in Pnc− children (table 1). At all time points, the
GMCs of anti-PsaA were 3–4 times higher in Pnc⁺ than in Pnc⁻ children (P < .001, all comparisons), in whom GMCs remained near the detection limit. The GMCs of anti-Ply increased in both Pnc⁺ and Pnc⁻ children but were more pronounced in the former group. The GMCs of anti-Ply were significantly higher in Pnc⁺ than in Pnc⁻ children at all time points (P < .05–.001). The GMC of anti-PspA increased slightly with age in Pnc⁺ children and remained constant or decreased in Pnc⁻ children; the difference between Pnc⁺ and Pnc⁻ children was significant at all time points (P < .05–.001).

When we compared Pnc⁺ carriers and Pnc⁺ sick children, there were only small differences in the proportions of samples positive for anti-PsaA, anti-Ply, and anti-PspA at different time points. At several times, the Pnc⁺ sick children had higher proportions of antibody-positive samples and higher GMCs than the Pnc⁺ carriers, but these differences were not statistically significant.

Representative examples. Figure 3 illustrates the association of Pnc⁺ culture findings with salivary IgA anti-PsaA, anti-Ply, and anti-PspA concentrations in 5 children in each category. In the Pnc⁻ children (panel 1), most antibody concentrations re-
maintained unchanged or decreased during follow-up. In the Pnc−
children (panels 2 and 3), Pnc− culture findings were asso-
ciated with a salivary antibody response in many but not all cases: a
Pnc− culture finding was not always followed by a salivary anti-
body response, and a salivary antibody response was not always
preceded by a Pnc− culture finding. In several cases, the anti-
PsaA and anti-Ply responses seemed to occur simultaneously and
with similar intensity. Anti-PspA responses were seen only oc-
casionally and often occurred later than the anti-PsaA and anti-
Ply responses.

Discussion

Our results show that IgA antibodies to the 3 pneumococcal
proteins (PsaA, Ply, and PspA) are common in saliva of children
and adults. These antibodies are probably produced locally, as
evidenced by the significant correlation of the IgA with the
presence of SC in anti-PsaA, anti-Ply, and anti-PspA. The anti-
body concentrations increased by age, and the increase was
associated with Pnc history. There were larger proportions of
IgA-positive samples and clearly higher mean antibody con-
centrations in the saliva of children in whom Pnc were found
by culture of NPS, NPA, or MEF samples than in children
without positive Pnc culture findings. However, the higher anti-
body values in the Pnc+ children seemed to be largely inde-
pendent of whether Pnc was found during asymptomatic car-
riage or illness, although a tendency for higher concentrations
was observed in the sick children. This might indicate a longer
or more intense contact with the bacteria during illness. The
Figure 3. IgA anti-pneumococcal surface adhesin A (PsaA), anti-pneumolysin (Ply), and anti-pneumococcal surface protein A (PspA) concentrations in saliva of 5 children: pneumococcus negative (Pnc⁻; panel 1), Pnc-positive (Pnc⁺) carriers (panel 2), and Pnc⁺ sick children (panel 3). Symbols on X-axis: C, Pnc⁻ nasopharyngeal swab culture; N, Pnc⁻ nasopharyngeal aspirate culture; M, Pnc⁺ middle ear fluid culture at ages indicated.
corresponding IgG antibodies to these same 3 proteins also are produced in serum of young children, and the antibody production is dependent on Pnc culture findings; however, no difference has been found between Pnc+ sick children and Pnc− healthy carriers [35, 36]. Some dissimilarities were noticed in the production of the IgA antibodies to the 3 protein antigens. Anti-PsaA and anti-Ply antibodies were detected in saliva of children by age 6 months, whereas anti-PspA antibodies appeared in saliva at a later age and in fewer children. Throughout the follow-up to age 2 years, the percentage of anti-PspA−positive samples was lower in both anti-PsaA− and anti-Ply−positive samples. A similar lower proportion of IgG anti-PspA antibodies also was seen in serum samples of these children [33]. The correlation between the salivary anti-PsaA and anti-Ply concentrations was stronger than that of either with anti-PspA. In adults, most samples were anti-PspA positive, and the proportion of samples containing anti-PspA (15 of 17) was only slightly lower than the proportion of anti-PsaA− and anti-Ply−positive samples (17 of 17). These findings, together with the clearly higher anti-PspA GMCs of adults, compared with those of children at age 24 months, suggest that the production of anti-PspA antibodies might mature only after this age and thus later than the other Pnc protein antigens assayed in this study. To our knowledge, no studies describing this kind of slow maturation of antibody response to a protein antigen have been reported. Another possible explanation for the smaller proportion of anti-PspA−positive samples might be the seroepidemiology of the colonizing Pnc. This particular antigen may be relatively rarely expressed among the Pnc colonizing the very young but may be frequently expressed in the Pnc colonizing adults. A similar slow induction of an IgG response to PspA in serum and weak mucosal antibody response to PspA in children colonized with Pnc was recently reported elsewhere [41].

In individual children, the salivary IgA anti-PsaA, anti-Ply, and anti-PspA responses were seen in association with the Pnc-positive culture findings (figure 3, panels 2 and 3). However, the relation was not always clear: increases in the salivary antibody concentrations were detected also in children with no Pnc detected (figure 3, panel 1). The Pnc cultures were done regularly at 1–6-month intervals (additional cultures were done during respiratory infections and AOM episodes). Thus, it is possible that these Pnc− children were Pnc+ at some time points but were not detected because of the study design.

Another possibility is that the anti-PsaA and anti-PspA responses might partly have been due to stimuli by other bacteria. Many Pnc surface antigens seem to be conserved, and several reports suggest shared amino acid sequences between Pnc and other streptococci of the normal flora. Thus, a significant degree of homology has been shown between the primary structure of Pnc PsaA protein and the putative lipoprotein adhesins of S. sanguis and S. parasanguis [42, 16]. In addition, the repeat domain of PspA possesses significant homology with the C-terminal repeat regions of certain proteins of S. mutans, S. dentisuperficiis, and Clostridium difficile [43]. However, this repeat domain of PspA was not present in the recombinant PspA antigen used to coat the ELISA plates in our study. The role of these shared, most probably immunogenic, epitopes in the natural development of mucosal and systemic immunity against Pnc clearly needs to be evaluated further. The samples collected in the FinOM Cohort Study will allow further studies.

In the present study, saliva samples were studied as a proxy for the mucosal immunology of the upper respiratory tract, in particular the nasopharynx. To our knowledge, no studies have evaluated the correlation between antibody concentrations of nasopharyngeal and saliva. Although antibody activity in the nasopharynx may be more relevant to Pnc, saliva collection is a noninvasive and easily repeatable sampling method. Thus, saliva collection is superior to such methods as nasopharyngeal washes, especially when repeated samples are obtained from infants or children. Our results show clear differences in the concentration of anti-Pnc IgA among exposed and apparently unexposed persons, which suggests that saliva serves as a proxy for local nasopharyngeal immune responses.

In conclusion, our results show that children can at an early age have considerable amounts of mucosal IgA anti-PsaA, anti-Ply, and anti-PspA antibodies and that these are associated with a history of Pnc found in NPS, NPA, or MEF samples. Even if the antibodies induced by natural encounters with Pnc would not prove sufficient for protection against Pnc, active immunization with the proteins (e.g., administered mucosally) might increase their protective activity. Encouraging results have been obtained in animal models. Both oral and intranasal immunization of mice with PsaA elicited protective immunity against Pnc carriage and systemic infection [44, 45]. Recently, intranasal immunization of mice with a mixture of PsaA and PspA proved to be highly protective against Pnc carriage [46]; the mixture of these 2 proteins elicited better protection than either alone. In the future, mucosal vaccination with Pnc proteins may be an alternative approach to current immunization strategies to prevent Pnc infections.

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