Do Antibodies to Pneumococcal Surface Adhesin A Prevent Pneumococcal Involvement in Acute Otitis Media?

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Antibodies to the pneumococcal (Pnc) surface protein PsaA are immunogenic and protective in experimental animal models, but their role in protection from Pnc disease in humans is not known. In the present study, the ability of antibodies to PsaA to prevent the progression of Pnc carriage to Pnc acute otitis media (Pnc AOM) was evaluated. Antibodies to PsaA were measured in acute-phase serum samples of children with AOM and with Streptococcus pneumoniae cultured from the nasopharynx. The risk of Pnc AOM was evaluated by a logistic regression model with anti-PsaA concentration as the predictive variable. Higher concentrations of antibodies to PsaA were associated with lower risk of the Pnc nasopharyngeal carriage progression to Pnc AOM. This was true in children 9–24 months old (odds ratio [OR], 0.49; 95% confidence interval [CI], 0.31–0.78) but not in children <9 months old (OR, 0.81; 95% CI, 0.48–1.35).

Otitis media (OM) is one of the most common childhood diseases; 0.5 million cases of acute OM (AOM) occur annually in Finland, which has a population of 5 million [1]. In the United States, ~24.5 million visits are made each year to physicians for the treatment of AOM [2]. *Streptococcus pneumoniae* is the most frequent bacterium in acute and recurrent otitis media but is also commonly carried in the nasopharynx of healthy persons. The asymptomatic carrier state may proceed, by means of transition mechanisms that are still incompletely understood, to symptomatic pneumococcal (Pnc) disease (e.g., Pnc AOM).

Pnc polysaccharide (PS) vaccine trials conducted in the 1970s for the prevention of AOM were disappointing [3]. Increased immunogenicity has since been achieved by conjugation of the capsular PS antigens to a protein carrier [4, 5]. In recent trials in the United States and Finland, the Pnc conjugate vaccine was highly efficacious against invasive Pnc disease and, moderately so, against Pnc AOM caused by vaccine serotypes [6, 7]. In addition, Pnc conjugate vaccine reduces the nasopharyngeal carriage of Pnc serotypes included in the vaccine but results in increased nasopharyngeal colonization with nonvaccine serotypes [8, 9]. Recently, similar replacement by nonvaccine serotypes was reported in AOM [7]. Furthermore, even with a limited number of serotypes included in the Pnc conjugate vaccine, this is a complex and expensive formulation for worldwide use. In contrast, many Pnc proteins are common to all strains. Protein antigens are expected to be T cell dependent and therefore already immunogenic in infancy. On this basis, such Pnc protein antigens have been investigated as vaccine candidates.

PsA, a 37-kDa Pnc surface protein, is immunogenic and protective against Pnc infection in experimental animal models [10–12]. Previous studies have shown that PsaA is highly species specific and genetically highly conserved [13–15]. Antibodies to PsaA are found in human sera, and the anti-PsaA concentration of children is related to previous Pnc contacts [16]. Recently, we showed an increase of anti-PsaA concentration between acute- and convalescent-phase serum samples in association with Pnc AOM [17]. However, the role of antibodies to protein antigens in protection against human Pnc disease is not known.

In this study, we sought evidence for the ability of antibodies to PsaA to protect against Pnc AOM. To eliminate as many confounding factors as possible, we focused on children with their first episode of AOM who at the same time carried *S. pneumoniae* in their nasopharynx. Because we knew that *S. pneumoniae* would be cultured from the middle-ear fluid (MEF) in about half of these children [18, 19], we asked whether the anti-PsaA concentration in the acute-phase serum sample would be a determinant of the risk of involvement of *S. pneumoniae* in the ear infection.
Materials and Methods

Study population and samples. The study population was selected from a cohort of 329 children enrolled in the Finnish Otitis Media (FinOM) Cohort Study [20]. These children were followed up from ages 2 through 24 months, to evaluate epidemiology and risk factors for AOM. Parents brought their child to the study clinic whenever they suspected AOM or the child needed medical care for acute respiratory infection. If AOM was diagnosed, myringotomy was performed, and an MEF sample was obtained. In addition, nasopharyngeal aspirate (NPA) and acute-phase serum samples were obtained during that visit. The study population comprised a subset of 94 children who had AOM that fulfilled the following criteria: the child’s first AOM diagnosis, available MEF and acute-phase serum samples, and concurrent NPA culture positive for *S. pneumoniae* (figure 1).

Laboratory methods. The NPA and MEF specimens were cultured immediately on selective blood agar plates (containing 5 µg/mL of gentamicin) and were incubated at 36°C with 5% CO₂ overnight at the study clinic. *S. pneumoniae* was identified by standard methods [21] and was serotyped, as described elsewhere [22]. NPA samples were homogenized and diluted in the virology laboratory at the Finnish National Public Health Institute in Helsinki. Rhinovirus detection was done by culture of the sample on HeLa cells, which was followed by reverse-transcription polymerase chain reaction [23]. Respiratory syncytial virus, influenza virus A, parainfluenza viruses 1, 2, and 3, and adenovirus antigens were detected by means of a solid-phase immunoassay based on monoclonal antibodies and time-resolved fluoroimmunoassay, as described elsewhere [23]. PsaA protein for this study was purified from a recombinant constructed by amplification of the gene from serotype 2 strain D39 [24]. IgG class anti-PsaA antibodies were measured by EIA with recombinant His-tagged PsaA protein, as described elsewhere [16].

Statistical methods. We report antibody concentrations as geometric mean concentrations (GMCs; U/mL) with 95% confidence intervals (CIs). We used the Student’s *t*-test to compare log-transformed antibody concentrations among groups of children. A logistic regression model was used to evaluate antibody concentrations (logarithm base e of anti-PsaAs) as a risk factor for Pnc involvement in AOM. The results of this model are expressed as odds ratio (OR) estimates for an increase of 1 log base e unit in antibody concentration. The scatter smoother in figure 2 were calculated by using Friedman’s SuperSmooother (S-Plus; MathSoft) [25].

Results

During follow-up from ages 2 to 24 months, 198 of 329 children had at least 1 AOM episode with MEF and acute-phase serum samples available (figure 1). Of these 198 AOM episodes, 177 were the first AOM event, and, in 94 children, the NPA sample was positive for *S. pneumoniae*. These 94 children (age range at diagnosis, 49–743 days; median, 273 days) formed our study population. MEF cultures were positive for *S. pneumoniae* in 44 (Pnc AOM) and were negative in 50 (non-Pnc AOM) events. These 2 groups did not differ in age, distribution of Pnc serotypes, or viral findings in the NPA samples or in known risk factors for AOM (day-care attendance or presence of siblings; table 1).

Figure 2 shows the acute-phase anti-PsaA concentrations in

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**Figure 1.** Finnish otitis media (FinOM) study population. AOM, acute otitis media; MEF, middle-ear fluid; NPA, nasopharyngeal aspirate.
Figure 2. Scatter plot and smoothed curves for age (months) and anti-PsaA concentration (U/mL) in acute-phase serum samples of children with first pneumococcal (Pnc) or non-Pnc acute otitis media (AOM), as identified by culture of middle-ear fluid. All children were nasopharyngeal carriers of Streptococcus pneumoniae.

The serum samples of children with Pnc AOM or non-Pnc AOM (plotted against age). Until age 9 months, the spectrum of anti-PsaA concentrations was similar in children with Pnc and non-Pnc AOM. Thereafter, the anti-PsaA concentration was lower among the children with Pnc AOM than in those with non-Pnc AOM. Thus, S. pneumoniae often caused the AOM, if the anti-PsaA concentration was low. Because the significance of anti-PsaA antibodies seemed to change abruptly at about age 9 months, the 94 children were further divided into groups as those <9 months old at the time of their first AOM and those ≥9 months old. Overall, the 47 children who were <9 months old had higher anti-PsaA GMCs than those who were older (107.9 vs. 38.8 U/mL; P = .001). Of the younger children, in the 19 with Pnc AOM, the anti-PsaA GMC was 91.4 U/mL, compared with 120.9 U/mL for the 28 children with non-Pnc AOM (P = .4). In the older group, the GMC of anti-PsaA was 18.3 U/mL in the 25 children with Pnc AOM and 91.3 U/mL in the 28 children with non-Pnc AOM (P = .001; table 2).

The risk of involvement of S. pneumoniae in AOM was further evaluated by a logistic regression model with the acute-phase anti-PsaA concentration as the predictive variable. Among the 47 children ≥9 months old, higher anti-PsaA concentration was associated with lower risk of Pnc AOM (OR, 0.49; 95% CI, 0.31–0.78). No such association was seen among children <9 months old (OR, 0.81; 95% CI, 0.48–1.35).

Discussion

In the present study, we asked whether antibodies to the Pnc protein PsaA could be a determinant of the risk of involvement of S. pneumoniae in AOM in children <24 months old. To exclude the possible confounding effect of the antibodies on Pnc transmission, we studied children who were nasopharyngeal carriers of S. pneumoniae and were seen at the study clinic because of their first episode of clinically diagnosed AOM. Among these 94 children, the serum anti-PsaA concentration was measured at the time of diagnosis and was tested in a logistic regression model as the predictive variable for Pnc involvement in the ear infection. Higher anti-PsaA was indeed associated with lower risk of Pnc AOM (OR, 0.49; 95% CI, 0.31–0.78) in the 47 children who were ≥9 months old at the time of the AOM. The known risk factors for AOM and for Pnc transmission, such as day-care attendance and presence of siblings [26–28], did not affect this risk.
We studied only "natural" antibodies, that is, antibodies produced early in life as response to contact with antigenic stimuli provided by components of the normal microflora. We previously showed that both Pnc carriage and Pnc infection (AOM) can act as the natural stimulus for antibodies to Pnc proteins, including PsaA, Pnc surface protein PspA, and pneumolysin [16].

In this study, we focused on anti-PsaA, because these are the most commonly observed antibodies in this age group; however, the correlation between antibodies to all 3 Pnc proteins is strong [16]. Thus, the association shown here between risk of Pnc involvement in AOM and anti-PsaA concentration should be interpreted with caution, since it is likely that the same association would hold for antibodies to other Pnc proteins, all produced as a response to the same stimulus. The epidemiological evidence could not separate the effect of the different anti–protein antibodies. In addition, young children produce salivary IgA class antibodies to PsaA [29]. Local antibodies may play a role in the protection from mucosal infections, such as AOM. In the present study, because saliva samples were not collected during an AOM event, we could not evaluate such possible protection. Nevertheless, our data suggest a protective effect of anti-Pnc protein antibodies, since the pattern of natural development of antibodies to the capsular polysaccharide that is known to be protective [6, 7] is very different and type specific [30].

In the interpretation of these findings, it is important to remember that all study children had AOM in addition to carrying S. pneumoniae in their nasopharynx. Therefore, it was not possible to evaluate the role of anti-PsaA in the prevention of clinical disease (AOM). However, we showed an association between anti-PsaA antibodies and reduced involvement of S. pneumoniae in AOM. In addition to its theoretical interest as the first demonstration of an association of these antibodies in an important human disease, this effect also has clinical relevance, since Pnc AOM is a clinically more severe disease that often leads to complications than non-Pnc AOM.

To date, all data indicating that antibodies to Pnc proteins might protect against Pnc carriage and disease have come from animal models. In mice, vaccination with PsaA elicited protection against nasopharyngeal carriage of S. pneumoniae [31] and, in combination with another Pnc protein PspA, against Pnc bacteremia and pulmonary Pnc infection [31]. The present study is the first to show that antibodies to Pnc proteins might offer

Table 1. Concomitant factors (pneumococcal [Pnc] serotypes and viral findings) in the nasopharyngeal aspirate (NPA) samples (day care attendance and presence of siblings) and age of the 94 children in this study.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Pnc AOM (n = 44)</th>
<th>Non-Pnc AOM (n = 50)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pnc serotype in NPA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19F</td>
<td>9</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>23F</td>
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<td>7</td>
<td>14</td>
</tr>
<tr>
<td>6A</td>
<td>6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>6B</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
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<tr>
<td>15</td>
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<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Others</td>
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<td>16</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
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<td>52b</td>
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<tr>
<td>Virus in NPAb</td>
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<tr>
<td>Rhinovirus</td>
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<tr>
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<td>6</td>
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<td>8</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Adenovirus</td>
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</tr>
<tr>
<td>Parainfluenza type 2</td>
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<td>1</td>
</tr>
<tr>
<td>Total</td>
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<td>26</td>
<td>49</td>
</tr>
<tr>
<td>Day-care attendance</td>
<td>11</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>With siblings</td>
<td>28</td>
<td>35</td>
<td>63</td>
</tr>
<tr>
<td>Age, days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>288</td>
<td>264</td>
<td>273</td>
</tr>
<tr>
<td>Range</td>
<td>68–577</td>
<td>49–743</td>
<td>49–743</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of children. AOM, acute otitis media.  
* Two children had 2 Pnc serotypes in the NPA sample.  
b Culture and virus-specific polymerase chain reaction for rhinovirus; virus antigen detection for the others.

Unexpectedly, a similar association between anti-PsaA and Pnc involvement in the AOM was not found among children <9 months old, which suggests a basic difference between the age groups. An obvious difference is the higher susceptibility to AOM of younger children due to undefined anatomic, physiologic, and/or immunologic factors that could overwhelm the potential protective effect of anti-PsaA. Our decision to study only the first AOM event of each child to preserve independence of all data points inevitably introduced a selection bias toward children less susceptible to AOM among the older children, who by definition had been spared AOM up to age 9–24 months. It seems quite plausible that the protection afforded by anti-PsaA would be relatively less efficient among the more susceptible younger children.

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Table 2. No. of children <9 or ≥9 months old with pneumococcal (Pnc) acute otitis media (AOM) or non-Pnc AOM and the geometric mean concentration (GMC) of antibodies to PsaA at the time of the child's first AOM event.

<table>
<thead>
<tr>
<th>AOM</th>
<th>&lt;9 months</th>
<th>≥9 months</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n GMC, U/mL (95% CI)</td>
<td>n GMC, U/mL (95% CI)</td>
<td>n GMC, U/mL (95% CI)</td>
</tr>
<tr>
<td>Pnc AOM</td>
<td>19 91.4 (43.2–193.2)</td>
<td>25 18.3* (10.3–32.6)</td>
<td>44 36.6b (22.1–60.7)</td>
</tr>
<tr>
<td>Non-Pnc AOM</td>
<td>28 120.9 (88.8–164.6)</td>
<td>22 91.3 (45.3–183.9)</td>
<td>50 106.8 (76.0–150.2)</td>
</tr>
<tr>
<td>Total</td>
<td>47 107.9c (76.8–151.6)</td>
<td>47 38.8 (23.7–63.5)</td>
<td>94 64.7 (47.4–88.4)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval.  
* Significantly lower than in non-Pnc AOM children (P < .001, Student's t test).  
† Significantly lower than in non-Pnc AOM children (P = .001, Student's t test).  
‡ Significantly higher than in children ≥9 months old (P = .001, Student's t test).
Protections against the development of human Pnc disease. On this basis, further research on antibodies to PsaA and other Pnc proteins as potential vaccine candidates is warranted.

Acknowledgments

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References