Intranasal Immunization of Mice with PspA (Pneumococcal Surface Protein A) Can Prevent Intranasal Carriage, Pulmonary Infection, and Sepsis with Streptococcus pneumoniae

Hong-Yin Wu, Moon H. Nahm, Y. Guo, Michael W. Russell, and David E. Briles

Many pathogens, including Streptococcus pneumoniae, are carried asymptptomatically on the nasopharyngeal mucosa and spread among individuals by close contact. Clinical disease results when pneumococci escape from the mucosa and invade sterile sites. Although systemic immunity can prevent invasive disease, control of person-to-person spread is probably dependent on immunity acting at the mucosal surface. Intranasal immunization of mice with PspA (pneumococcal surface protein A) or a capsular 6B polysaccharide–tetanus toxoid conjugate induced mucosal and systemic antibody responses and provided long-lasting protection against carriage of S. pneumoniae. Resistance to carriage was dependent on mucosal rather than systemic immunity and was effective against heterologous strains of heterologous PspA types. Intranasal immunization with PspA also protected against systemic infection following intravenous, intratracheal, and intraperitoneal challenge.

The majority of infectious diseases directly affect or are acquired through the mucosal surfaces. For many organisms, colonization of the respiratory, gastrointestinal, and urogenital tracts is the first step in pathogenesis, and prevention of colonization should effectively block both transmission and infection. Specific immune protection of mucosal surfaces is mediated by the mucosal immune system and by the small amounts of serum immunoglobulin that reach these sites. The most conspicuous product of the mucosal immune system is secretory IgA. Generation of specific secretory IgA antibodies usually requires stimulation of specialized inductive sites of the mucosal immune system. Only in recent years have effective strategies for immunization at these sites been developed [1], and the development of mucosal vaccines has lagged behind conventional parenteral vaccination [2].

Streptococcus pneumoniae cause pneumonia, meningitis, otitis media, ocular infections, bacteremia, and sinusitis in humans and are a major cause of fatal infections worldwide [3–5]. Capsular polysaccharide (CPS) [6, 7] and pneumococcal surface protein A (PspA) [8] are both virulence factors in pneumococci. Polysaccharide (PS) is antiphagocytic and is the most conspicuous protection-eliciting antigen of pneumococci. The ability of serum antibodies to CPS to provide serotype-specific protection is well established [9]. Since children <2 years of age respond poorly to immunization with PS vaccines [10], protein conjugates of PS are being investigated as a potential human vaccine for children [11]. There are >90 different CPSs constituting 48 non–cross-reactive PS groups [12]. Thus, to be effective, PS vaccines must contain multiple PS [13].

Pneumococci are acquired through aerosols or by direct contact and first colonize the upper airways, where they can be carried for weeks or months. Some 10%–20% of adults carry pneumococci in the upper nasopharynx at any time; colonization rates in young children and in the elderly are much higher. In most cases, colonization does not result in apparent disease [14, 15] and even highly virulent strains can colonize without causing disease [16, 17].

PspA is necessary for full virulence of pneumococci and retards their clearance from the blood of mice [8, 18]. Antibody to PspA facilitates clearance and protects against death [8, 19, 20]. Like CPS, PspA is variable in structure. PspA from different isolates usually differ in molecular weight, the combination of epitopes expressed [21–23], and pspA-associated restriction fragment length polymorphism patterns [24]. However, PspAs share many cross-reactive epitopes, and immunization with a single PspA is cross-protective in mice against fatal infection with strains of many different capsular types [8, 21, 25, 26].

Whether parenteral immunization with pneumococcal PS or PS-protein conjugates can reduce pneumococcal carriage in humans is still under investigation [27, 28]. Much like Haemophilus influenzae, immunization with serotype b PS protein conjugates reduced carriage rates from ~4% to <1% [29, 30]. Although immunity at the nasal mucosa can be elicited through...
Methods

Colony-forming units (cfu) [32]. Inoculation intranasally delivered 10^6 S. pneumoniae to evaluate its ability to block intranasal (inl) or intratracheal (intratracheal [int]) acquired PS. PS-specific antibody assays were done in the presence of pneumococcal PS-immunized mice intranasally (inl) with isolated PspA [32] to mined by ELISA using plates coated with isolated PspA or 6B-PS as antigen. Antibody levels were determined by ELISA using plates coated with isolated PspA or 6B-PS. PS-specific antibody assays were done in the presence of pneumococcal CPS to prevent the detection of any antibody to cell wall CPS [38]. Isotype specific antibody levels were determined by using alkaline phosphatase- or peroxidase-conjugated antibodies specific for mouse immunoglobulin isotypes [37, 38].

Mouse challenge. Mice were challenged inl 2 weeks or 144 days after the last immunization. Other mice were challenged int, intravenously (iv), or ip 4 weeks after the last injection. Challenges ip and iv were done as previously described using log-phase pneumococci diluted in Ringer’s lactate to the indicated number of colony-forming units (cfu) [32]. Inoculation int delivered 10^6 L of Ringer’s solution containing cfu into the nares of nonanesthetized mice. We did inoculation int by a nonsurgical procedure that used a 69-mm finely drawn gel-loading plastic pipette tip (05-1000, Becton Dickinson). CTBs greatly augment immune responses to protein antigens given inl [33].

Table 1. Characteristics of encapsulated S. pneumoniae studied.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Capsule</th>
<th>Serotype</th>
<th>LD_{50} in BALB/c and (CBA/N) mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intravenous</td>
</tr>
<tr>
<td>A66</td>
<td>3</td>
<td>13</td>
<td>10^6 (&lt;10^7)</td>
</tr>
<tr>
<td>L82016</td>
<td>6B</td>
<td>33</td>
<td>&gt;10^7 (&gt;10^8)</td>
</tr>
<tr>
<td>BG7322</td>
<td>6B</td>
<td>24</td>
<td>10^7 (&lt;10^8)</td>
</tr>
<tr>
<td>BG8826</td>
<td>23F</td>
<td>6</td>
<td>&gt;10^7 (10^8)</td>
</tr>
</tbody>
</table>

* Collected by B. M. Gray, University of Alabama at Birmingham.

the common mucosal pathway by immunization at other mucosal sites, mucosal immunity is often strongest at or near the site of immunization [31].

Since pneumococci colonize the nasopharynx and infect the lungs and upper airway, we investigated the ability of mucosal immunizations to block infection and colonization at these sites. When pneumococci spread from the nasopharynx to the lung, they must pass through bronchial tissue. Thus, mucosal immunity at both the nasopharynx and bronchial sites might play a significant role in preventing pulmonary infection. We used a 69-mm finely drawn gel-loading plastic pipette tip (05-1000, Becton Dickinson) for mouse challenge [34]. The only other strain used was S. pneumoniae strain R36A, which has been used for isolation of PspA. This mock immunization blocks PspA production. The resulting material contained essentially no protein, since it lacked PspA, but was administered at the same dilution (based on the starting volume of bacterial culture) as the material from strain R36A [32]. This mock immunization always gave results identical to those obtained with CTB alone (data not shown). 6B-PS was prepared by the method of Schneerson et al. [36] and contained 3 μg of TT per 2 μg of 6B-PS. Mice were bled 10 days after their last immunization. Saliva (~500 μL) was collected from mice 10 days after the last immunization by pipe fitting the plastic tip [37]. Antibody levels were determined by ELISA using plates coated with isolated PspA or 6B-PS. PS-specific antibody assays were done in the presence of pneumococcal CPS to prevent the detection of any antibody to cell wall CPS [38]. Isotype specific antibody levels were determined by using alkaline phosphatase- or peroxidase-conjugated antibodies specific for mouse immunoglobulin isotypes [37, 38].

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Bacterial strains. Table 1 lists S. pneumoniae strains that we used for mouse challenge [34]. The only other strain used was R36A, an avirulent rough variant of capsular type 2 strain D39 [8, 35], which has been used for isolation of PspA.

Immunizations, antigens, and ELISAs. Mice received three immunizations inl 10 days apart. On each occasion, 150 μg of PspA or 1500 ng of a conjugate of 6B-PS with tetanus toxoid (6B-TT) in 10–12 μL of Ringer’s lactate was slowly delivered into the nares. CTB (4–5 μg; List Biological Laboratories, Campbell, CA) was included in the first two immunizations. Other mice received two systemic injections of 1 μg of PspA: The first was given subcutaneously with complete Freund’s adjuvant (CFA); the second (20 days later) was given intraperitoneally (ip) without adjuvant. PspA for immunization was isolated from R36A pneumococci on choline-Sepharose columns as described previously [32]. For some control immunizations, we followed the same isolation procedure using strain WG44.1, which contains a mutation that blocks PspA production. The resulting material contained essentially no protein, since it lacked PspA, but was administered at the same dilution (based on the starting volume of bacterial culture) as the material from strain R36A [32]. This mock immunization always gave results identical to those obtained with CTB alone (data not shown). 6B-PS was prepared by the method of Schneerson et al. [36] and contained 3 μg of TT per 2 μg of 6B-PS. Mice were bled 10 days after their last immunization. Saliva (~500 μL) was collected from mice 10 days after the last immunization by pipe fitting the plastic tip [37]. Antibody levels were determined by ELISA using plates coated with isolated PspA or 6B-PS. PS-specific antibody assays were done in the presence of pneumococcal CPS to prevent the detection of any antibody to cell wall CPS [38]. Isotype specific antibody levels were determined by using alkaline phosphatase- or peroxidase-conjugated antibodies specific for mouse immunoglobulin isotypes [37, 38].

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Nasal wash samples were collected from mice after sacrifice. The trachea was cut at the top of the larynx, and 50 μL of Ringer’s solution was injected and collected from the tip of the nose. The bacteria were plated on blood-agar plates containing 4 μg/mL gentamicin to inhibit the growth of most nonpneumococcal bacteria that might be present [39]. To further ensure that the bacteria observed were pneumococci, samples were also plated on a second set of gentamicin-containing plates that also contained 5 μg/mL optochin (ethyl hydrocupreine hydrochloride; Sigma, St. Louis).
In general, there were few if any colonies on the blood agar plates containing gentamicin plus optochin, confirming that the bacteria observed on the gentamicin-containing plates were pneumococci. In the rare cases in which there were >15% cfu on the gentamicin plus optochin plates, data from that mouse were discarded. In some initial experiments, colonization of the inoculated pneumococci was also confirmed by capsular typing of the pneumococci recovered from the nasal tissues.

**Statistical analysis.** We expressed antibody levels as geometric means with the SE factor (the number by which a number must be multiplied or divided to obtain the upper or lower SE limits). Statistical differences were calculated with a one-tailed Wilcoxon two-sample rank test. P ≥ .05 was considered nonsignificant.

**Results**

**Immune response to immunization inl with PspA.** When given inl with CTB, 150-ng doses of PspA elicited significant IgA antibodies in saliva of BALB/cJ mice (table 2). No IgG antibody to PspA was detectable (<0.01 µg/mL) after immunization inl. No antibody response to PspA was observed in the absence of CTB (table 2) or if the dose of PspA was reduced to 15 ng (data not shown). Immunization inl with PspA and CTB elicited serum IgG and IgA antibody to PspA (table 3). Although parenteral immunization with 1000 ng of PspA in CFA induced a strong serum IgG antibody response, it failed to induce any serum IgA antibody to PspA and did not elicit detectable anti-PspA of any isotype in saliva (tables 1, 2). Immunization of CBA/N mice using similar intranasal and parenteral protocols elicited essentially the same responses (data not shown) as obtained with BALB/c mice.

**Protection against invasive disease by immunization inl with PspA.** To test the ability of immunization inl with PspA to protect against pulmonary infection, ~100 LD₅₀ of A66 pneumococci were inoculated intratracheally (int) into BALB/c mice in 20 µL of Ringer’s solution. Protection against acquisition inl of pneumococcal infection was tested by inoculating immunized CBA/N mice inl with 3 × 10⁶ cfu of virulent A66 pneumococci. CBA/N, rather than BALB/c mice were used for challenge inl, because inoculation inl of A66 readily kills CBA/N mice but does not kill 100% of BALB/c mice, even when

### Table 2. Salivary IgA responses of BALB/c mice to R36A PspA and CTB.

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Route</th>
<th>Geometric mean µg/mL IgA (SE factor)</th>
<th>Geometric mean, % specific IgA (SE factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PspA + CTB</td>
<td>inl</td>
<td>0.9 (1.3)</td>
<td>14 (1.2)</td>
</tr>
<tr>
<td>PspA⁺ + CTB</td>
<td>inl</td>
<td>≤0.01 †</td>
<td>≤1 †</td>
</tr>
<tr>
<td>CTB</td>
<td>inl</td>
<td>1.0 (1.2)</td>
<td>≤0.01 §</td>
</tr>
<tr>
<td>PspA + CFA</td>
<td>sc/ip</td>
<td>≤0.01 †</td>
<td>≤0.01</td>
</tr>
<tr>
<td>CFA</td>
<td>sc/ip</td>
<td>≤0.01 †</td>
<td>≤0.01</td>
</tr>
</tbody>
</table>

* Each group contained 5 mice. PspA⁺ refers to immunization preparation for mice containing PspA⁺ strain. Immunizations used cholera toxin B subunit (CTB) or complete Freund’s adjuvant (CFA) as adjuvants. inl, intranasally; ip, intraperitoneally; sc, subcutaneously.

² Antibody (10 days after last immunization) to CTB was assayed using microtitration plates coated with cholera toxin (CT).

Statistical significance from PspA⁺ corresponding adjuvant by Wilcoxon 2-sample rank test: P < .0001, †.01, §.05.

### Table 3. Serum antibody responses of BALB/c mice to R36A PspA and CTB.

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Route</th>
<th>Geometric mean µg/mL anti-PspA (SE factor)</th>
<th>Geometric mean µg/mL anti-CTB (SE factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PspA + CTB</td>
<td>inl</td>
<td>15 (1.2)*</td>
<td>4073 (1.1)</td>
</tr>
<tr>
<td>PspA⁺ + CTB</td>
<td>inl</td>
<td>≤0.5</td>
<td>≤0.5</td>
</tr>
<tr>
<td>CTB</td>
<td>inl</td>
<td>≤0.5</td>
<td>6450 (1.2)</td>
</tr>
<tr>
<td>PspA + CFA</td>
<td>sc/ip</td>
<td>291 (1.7)*</td>
<td>≤0.01</td>
</tr>
<tr>
<td>CFA</td>
<td>sc/ip</td>
<td>≤0.5</td>
<td>≤0.01</td>
</tr>
</tbody>
</table>

**NOTE.** PspA⁺ refers to immunization preparation for mice containing PspA⁺ strain. Immunizations used cholera toxin B subunit (CTB) or complete Freund’s adjuvant (CFA) as adjuvants. inl, intranasally; ip, intraperitoneally; sc, subcutaneously. Statistical significance from PspA⁺ corresponding adjuvant by Wilcoxon 2-sample rank test: P < *.01, †.05.
Figure 1. Protection against fatal challenge by intranasal (i.n.) immunization with R36A PspA adjuvanted with cholera toxin subunit B (CTB). Statistical comparisons of PspA + CTB vs. respective CTB-only controls are by Wilcoxon 2-sample rank test. A, Intratracheal (i.t.) challenge of BALB/c mice with $5 \times 10^5$ A66 ($\sim 100\times LD_{50}$) S. pneumoniae 14 or 144 days after i.n. immunization. $\geq 20$ mice/group 2 weeks after immunization; 5 PspA immune and 10 CTB only mice 5 months after immunization. B, i.n. challenge of CBA/N mice with $3 \times 10^6$ cfu of A66 S. pneumoniae 30 days after i.n. immunization with PspA adjuvanted with CTB. PspA + CTB vs. CTB, $P = .011$. C, Intravenous (i.v.) challenge of BALB/c mice with $8 \times 10^4$ cfu A66 ($10\times LD_{50}$) S. pneumoniae 2 weeks after i.n. immunization with PspA adjuvanted with CTB. PspA + CTB vs. CTB, $P = .045$. D, Intraperitoneal (i.p.) challenge of BALB/c mice with 1750 cfu A66 ($\sim 90\times LD_{50}$) S. pneumoniae 2 weeks after i.n. immunization. PspA + CTB vs. CTB only, $P = .004$.

$10^5$ cfu are inoculated. Immunization i.n. with PspA provided good protection against challenge both i.n. and i.t. (figure 1A, B). Challenge of mice i.t. 144 days after immunization elicited long-term immunity (figure 1A).

Mice immunized i.n. with PspA and CTB were also protected against sepsis and bacteremia; 3 days after challenge i.t., immune mice had $\leq 30$ cfu/mL in blood, whereas CTB control mice had $10^4$–$10^6$ cfu/mL (data not shown). Challenge i.v. directly tested for protection against sepsis. Immunization i.n. with PspA from strain R36A protected BALB/c mice against otherwise fatal challenge i.v. with S. pneumoniae A66 capsular type 3 (figure 1C).

Although inoculation i.p. is not a natural route of pneumococcal infection, it has been widely used as a model for passive immunity. Fewer pneumococci are needed to kill mice i.p. than by other challenge routes [40]. Previous studies have shown that subcutaneous immunization of mice with PspA can protect against death following challenge i.p. with S. pneumoniae [32]. In the present studies, we observed that even though immunization i.n. used $< 1/6$ the amount of PspA used in the subcutaneous
Thus, although mucosal immunity may contribute to protection of 6B-TT had the fewest pneumococci in the nasopharynx.

With the fatal challenge (inl, iv, int, or ip) challenge and illustrate cross-protection, since challenge strain A66 is PspA type 13. PspA types are determined from patterns of reactivity with a panel of seven monoclonal antibodies. PspA serotypes 6, 13, and 24 differed from that of R36A PspA (serotype 25) by 4, 4, and 2 epitope reactivities, respectively [21].

Protection against nasopharyngeal carriage by immunization inl with CPS. Protein conjugates of pneumococcal PS are being investigated as a potential human vaccine [11]. To test the ability of PS-protein conjugates to protect against carriage, 2-month-old BALB/c mice were immunized inl with pneumococcal type 6B PS conjugated to 6B-TT in the presence of CTB. Mice immunized inl three times with 1.5 μg of conjugate produced readily detectable levels of antibody to 6B PS (geometric mean = 120 ng/mL; SE factor = 3.4) in sera but barely detectable levels in saliva (geometric mean = 2.8 ng/mL; SE factor = 2.0). Serum antibodies were almost exclusively IgG, but the only salivary antibodies detected were IgA. Mice immunized with three doses of 0.3 μg of conjugate generated unmeasurable levels of serum or saliva immunoglobulins to 6B PS. The highest levels of carriage were in the control mice given CTB only. Mice immunized with the highest dose of 6B-CTT had the fewest pneumococci in the nasopharynx (figure 2). The numbers of pneumococci recovered from the nasopharynx of mice immunized with 6B-CTT were significantly lower (P = .03) than those from control mice.

Discussion

We observed that immunization inl of mice with PspA was efficacious against otherwise fatal infection when challenged with pneumococci int or inl. For challenge int, protection lasted >5 months after immunization. Protection against fatal infection could have resulted from mucosal immunity, systemic immunity, or both. Systemic immunity to PspA can protect against pneumococcal sepsis [20, 32]. From the present studies, immunization inl with PspA resulted in protective immunity in the systemic (protection from challenge iv and ip) and mucosal (protection from carriage) compartments. It is thus likely that both mucosal and systemic immunity may have contributed to protection against fatal challenge inl and int.

To study immunity at the mucosal surface, we used a model in which carriage could be observed in the absence of sepsis. It should be noted that the ability of pneumococci to cause sepsis versus carriage only after challenge inl of mice is a function of both the mouse strain and the strain of pneumococci. In these studies fatal infections following inl inoculation...
Table 5. Ability of serotype 25 PspA from strain R36A to cross-protect CBA/N mice against carriage with two unrelated pneumococci.

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Challenge strain</th>
<th>Days before challenge(^\ast)</th>
<th>Carriage(^1)</th>
<th>median CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>PspA + CTB</td>
<td>BG- Capsule type</td>
<td>7322 6B 6 14 1 : 3 &lt;3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTB</td>
<td></td>
<td>14 3 : 1 1650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PspA + CTB</td>
<td>8826 23F 36</td>
<td>14 1 : 3 &lt;3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTB</td>
<td></td>
<td>14 3 : 1 1865</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PspA + CTB</td>
<td>8826 23F 36</td>
<td>144 4 : 4 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTB</td>
<td></td>
<td>144 8 : 0 1035</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. In 50-μL blood samples, all mice had <3 cfu. In all cases, there were <3 cfu of pneumococci/50 μL of blood at time of sacrifice.

\(^\ast\) Days between immunization and intranasal challenge with 10\(^7\) cfu in 12 μL.

\(^1\) Carriage evaluated as in table 4. Yes, >3 cfu; no, <3 cfu. For 14-day data, all \(P = .01\) by 1-tailed Wilcoxon test for PspA + CTB vs. all CTB only. For 144-day data, \(P = .0001\) for PspA + CTB vs. CTB.

were observed only when A66 pneumococci were used to inoculate the hypersusceptible CBA/N mice. Most strains of pneumococci, however, can cause carriage in mice without accompanying sepsis (unpublished data). The absence of sepsis in the carriage model was critical to our ability to determine that protection could act at the mucosal surface. Since mice carrying the pneumococci in the nasal tissues were not septic, it was clear that the pneumococci found in the nasopharynx were present because they had colonized and not just contaminated (a function of generalized sepsis).

The protection against carriage appeared to result from local rather than systemic immunity. For example, both the inl and the subcutaneous/ip immunization routes elicited systemic antibody responses, but only immunization inl elicited detectable antibody in secretions. Although it seems likely that the large amount of IgA antibody to PspA observed in the secretions plays a role in protection at mucosal sites, these data do not rule out the possibility that the much smaller (largely undetectable) levels of IgG in the secretions or some type of cell-mediated response may contribute to the resistance observed here.

Our demonstration that immunization inl with 6B-TT conjugate can reduce carriage of pneumococci indicates that immunity to antigens other than PspA can also protect against carriage. CPS, PspA, and IgA protease show the highest degree of serologic variation of any pneumococcal antigens [12, 21, 41]. Since the human reservoir of pneumococci is thought to be maintained primarily by nasopharyngeal carriage, it might be expected that the evolutionary selection that has driven serologic variation in CPS, PspA, and IgA protease acts at the level of carriage, rather than at systemic infection. Mouse IgA is not cleaved by pneumococcal IgA1 protease, and we did not attempt to assess the protection-eliciting role of this potential virulence factor in mice. In the case of immunity to PspA and capsule, however, protection against carriage was observed.

The ability of PspA and the 6B-TT conjugate to elicit protection against nasopharyngeal carriage in mice raises the possibility that mucosal immunity to these antigens might confer protection against carriage in humans. The observation that immunization with PspA can cross-protect against carriage strains bearing different PspAs enhances the prospects for the use of PspA as a mucosal vaccine. Moreover, in tests of the cross-protection elicited by individual PspA immunogens, the carriage model may have one advantage over the systemic infection model. Strains of many capsular types, including some of the major childhood capsular types 14, 19F, and 23F, are quite avirulent when injected into mice [34] but are readily carried in the nasopharynx (unpublished data).

Figure 2. No. of bacterial colonies recovered from mice immunized 3 times with 1.5 μg of 6B polysaccharide and tetanus toxoid (6B-TT) plus cholera toxin subunit B (CTB), 0.3 μg of 6B-TT plus CTB, or CTB alone. Dose refers to polysaccharide content of conjugate.
Protection against both inl carriage and challenge int was still apparent 5 months after immunization. This long duration of protection provides support for the hypothesis that mucosal immunity may play an important role in protection against pneumococcal colonization and disease. It is important to note, however, that the observations reported here have been made with mouse models of pneumococcal disease. Knowledge of the exact relevance of these data to immunity to human carriage and systemic pneumococcal disease must await detailed human studies. Nonetheless, the observation the PsPA can elicit long-lasting mucosal immunity to infection and carriage provides encouragement that this easily produced, highly immunogenic protein may be useful as a human vaccine. The observation that immunization inl with PsPA and 6B-TT can provide immunity to carriage strengthens arguments for testing mucosal immunization as a potential route for the delivery of pneumococcal vaccines in humans.

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References


