Immunization of Humans with Recombinant Pneumococcal Surface Protein A (rPspA) Elicits Antibodies That Passively Protect Mice from Fatal Infection with Streptococcus pneumoniae Bearing Heterologous PspA

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Pneumococcal capsular polysaccharide vaccines containing isolated polysaccharide are not efficacious in children <2 years old. This age group is a major target for pneumococcal vaccines [1]. The polysaccharide vaccine also protected <60% of elderly adults at risk for pneumococcal infection [2]. Recently, a 7-valent polysaccharide-protein conjugate vaccine has been shown to be immunogenic in young children and able to elicit protection against invasive infection [3]. However, pneumococci are highly diverse with respect to their capsular types [4, 5], and the polysaccharide-protein conjugate vaccines will not protect against strains of the many capsular types not included in the vaccine.

Although the 7-valent vaccine is likely to protect against 85%–90% of the strains that cause invasive infections in children in the United States, the coverage in other parts of the world will be as low as 43% because of regional differences in capsular types that cause infections in children [6, 7]. Failure to cover all polysaccharide types is an even greater problem with adults than with children, because clinically important infections in adults are caused by strains of a larger variety of capsular types than is the case for infections in children [8–10]. An additional concern is that the polysaccharide-protein conjugate vaccines will probably be too expensive for widespread use in the developing world, where 1–5 million children die each year of pneumococcal respiratory infection [8]. Moreover, the cost of the presently licensed vaccine is several-fold greater than the savings that health maintenance organizations in the United States will realize by using the vaccine [11].

Several proteins of Streptococcus pneumoniae have been proposed for use in human vaccines on the basis of their ability to elicit protective immunity in mice [12]. Of these, PspA has recently undergone a phase I clinical trial that found recombinant PspA (rPspA) to be safe and highly immunogenic in humans [13]. PspA is found on all pneumococci [14]. It is known to interfere with fixation of complement component C3 [15–17], thus potentially blocking downstream events that lead to opsonization and chemotaxis. PspA has been shown to bind lactoferrin, but the biological significance of this property is not known [18].

PspA has 3 major structural domains [19, 20]. The N-terminal, which is ~40% of the molecule, has a sequence consistent with a coiled-coil α helix. In the center of the molecule is a proline-rich region of ~60–80 amino acids. The C-terminal end of the molecule contains 9 or 10 repeats of 20 amino acids each. These repeats form a phosphocholine binding site [21]. Although PspA is structurally [20] and antigenically variable, antibodies to PspA are highly cross-reactive [13, 14] and cross-protective [22–24]. Mapping studies indicate that the major cross-protective epitopes reside in the ~100 amino acids of the α-helical region that is adjacent to the proline-rich region [25]. On the basis of the sequences of this 100–amino acid region, PspAs have been divided into 6 clades that constitute 3 families.
Human Antibody to PspA Protects Mice

Table 1. Characteristics of donors of pre- and postimmune serum in study of protection against pneumococcal infection by pneumococcal surface protein A (PspA) vaccine.

<table>
<thead>
<tr>
<th>Immunogen, patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Race</th>
<th>Pre IgG anti-PspA, μg/mL</th>
<th>Post IgG anti-PspA, μg/mL</th>
<th>Pre IgG anti-type 3 polysaccharide, μg/mL</th>
<th>Post IgG anti-type 3 polysaccharide, μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PspA + alum*</td>
<td>26</td>
<td>F</td>
<td>Asian</td>
<td>4.6</td>
<td>383</td>
<td>0.62</td>
<td>0.68</td>
</tr>
<tr>
<td>81</td>
<td>30</td>
<td>M</td>
<td>White</td>
<td>14.4</td>
<td>372</td>
<td>0.28</td>
<td>0.24</td>
</tr>
<tr>
<td>87</td>
<td>43</td>
<td>M</td>
<td>White</td>
<td>4.6</td>
<td>601</td>
<td>2.97</td>
<td>2.87</td>
</tr>
<tr>
<td>90</td>
<td>28</td>
<td>M</td>
<td>Asian</td>
<td>35</td>
<td>690</td>
<td>1.52</td>
<td>1.45</td>
</tr>
<tr>
<td>115</td>
<td>25</td>
<td>M</td>
<td>White</td>
<td>13.2</td>
<td>366</td>
<td>0.87</td>
<td>0.81</td>
</tr>
<tr>
<td>Alum</td>
<td>43</td>
<td>F</td>
<td>White</td>
<td>9.0</td>
<td>6.5</td>
<td>5.24</td>
<td>5.20</td>
</tr>
<tr>
<td>52</td>
<td>26</td>
<td>M</td>
<td>White</td>
<td>6.0</td>
<td>6.3</td>
<td>2.17</td>
<td>3.35</td>
</tr>
<tr>
<td>84</td>
<td>45</td>
<td>M</td>
<td>White</td>
<td>6.4</td>
<td>8.1</td>
<td>1.79</td>
<td>1.94</td>
</tr>
<tr>
<td>114</td>
<td>39</td>
<td>F</td>
<td>Black</td>
<td>3.7</td>
<td>4.2</td>
<td>1.56</td>
<td>1.63</td>
</tr>
</tbody>
</table>

NOTE. Immunizations were given to patients at days 0 and 30. Serum samples were drawn immediately before first injection (Pre) and on day 60 (Post). F, female; M, male.

* For patient 46, 25 μg; for all others, 125 μg.

PspAs of families 1 and 2 are found in about equal frequencies and collectively are expressed by >98% of strains [20].

Now that the PspA family and clade of many of our laboratory strains have been determined [20], a reexamination [26] of our previous studies of PspA immunity in mice [22–24] has shown that the PspA used for immunization and the PspA of the challenge strain need not be of the same clade or family for the elicited antibodies to be protective.

Indirect evidence suggests that human antibodies to PspA may be protective. Among children with invasive bacterial infections, those with the lowest titers of antibody to PspA are most frequently infected with pneumococci [27]. However, these results are only correlative. It is possible that the levels of antibody to PspA are directly correlated with levels of other protective pneumococcal antigens.

A direct test of the ability of human antibodies to PspA to protect mice from pneumococcal infection was not possible, because it has not been possible to remove anti-PspA antibodies from PspA-immunoadsorbent columns without denaturing them (authors’ unpublished data). Another way to test the protective capacity of human antibodies to PspA is to immunize humans with recombinant PspA and to compare the ability of the pre- and postimmune serum samples to protect mice from pneumococcal infection. The human antibody to PspA elicited in a recent trial with rPspA from strain Rx1 (rPspA/Rx1) has been found to be significantly cross-reactive with PspAs within families 1 and 2 [13]. These same serum samples were examined in the present study to determine whether human antibody to PspA can protect mice from otherwise fatal challenge with S. pneumoniae.

Methods

Immune serum samples. Pre- and postimmune serum samples (table 1) were obtained from 90 healthy adults immunized with a recombinant fragment comprising the N-terminal 314 amino acid of PspA from strain Rx1 (rPspA/Rx1) adsorbed to aluminum hydroxide adjuvant (alum) and from 30 adults immunized with alum alone [13]. Subjects were immunized on days 0 and 30 with rPspA/Rx1 on alum or with alum alone. Serum samples for this study were collected immediately before the day 0 immunization or 60 days after the day 0 immunization.

Five pairs of serum samples were chosen from subjects immunized with rPspA/Rx1, in which there were large differences in the levels of antibody to PspA in the pre- and postimmune serum samples. Four additional pairs of pre- and postimmune serum samples were selected from subjects immunized with alum alone. The latter serum samples were chosen to avoid any that had unusually high preimmune levels of antibody to PspA (table 1).

Assay of serum samples for antibody to PspA. The determination by ELISA of the levels of IgG antibody to PspA has been described elsewhere [13]. All of the anti-PspA levels reported here were determined elsewhere as part of the assessment of the antibody responses in the phase I trial of rPspA/Rx1 and the study of the cross-reactivity of human antibodies to PspA [13].

Assay of serum samples for antibody to polysaccharide. The amount of anti-type 3 polysaccharide antibody was determined by a sandwich ELISA [28], with some modifications. Plates (Polysorb; Nunc, Roskilde, Denmark) were coated with 5 μg/mL type 3 polysaccharide in PBS (American Type Culture Collection, Rockville, MD). Serum samples were preadsorbed with 10 μg/mL cell wall polysaccharide (Statens Seruminstitut, Copenhagen) and 10 μg/mL type 9V polysaccharide to neutralize antibodies to cell wall polysaccharide and non–cell wall polysaccharide contaminants in pneumococcal polysaccharide preparations [29]. Alkaline phosphatase–conjugated goat anti–human polyclonal immunoglobulins (Sigma, St. Louis) were used for the detection of bound antibodies, and p-nitrophenyl phosphate was used as a developing substrate. A serum pool (89-SF; obtained from C. Frasch, US Food and Drug Administration, Bethesda, MD) was used as the standard.

Challenge strains of S. pneumoniae. The different pneumococcal isolates were chosen to include mouse-virulent strains of both major PspA families. The capsular types, PspA clades, and PspA families of the challenge strains used are provided in table 2. All strains were selected for their virulence, for being capsular types 3, 6A, or 6B, and for the fact that they expressed a diversity of PspAs. All strains are mouse virulent by the intravenous route. Capsular type 3 strain A66.1 [31] is a mouse-passaged derivative.
of mouse-virulent strain A66, originally isolated by Avery et al. [30]. Strain 3JYP2670 was obtained from J. Yother (University of Alabama at Birmingham).

**Passive protection of mice with human serum samples.** Each pre- and postimmune serum sample pair were tested against the A66.1 challenge strain in a separate experiment containing 7 groups of 10 CBA/CAHN-XID/J mice (CBA/N mice). In each experiment, a known protection-eliciting serum pool from mice immunized with rPspA/Rx1 was included as a positive control and was always found to protect mice from challenge (data not shown). In each experiment, 4 groups were given 100 µL of postimmune serum diluted 1:25, 1:100, 1:400, or 1:1600 in lactated Ringer’s injection solution. Two groups were given 1:25- or 1:100-diluted preimmune serum. One group received Ringer’s injection solution alone. All diluted serum samples or Ringer’s injection solution alone was given intraperitoneally 1 h before intravenous challenge with pneumococci. Mice were challenged with the minimum number of pneumococci necessary to kill virtually all nonprotected mice. This was 30–100 times the LD50 of each strain used. Mice infected with capsular type 3, 6A, or 6B pneumococci were observed for 21, 41, and 41 days, respectively. Dates of all deaths were recorded. Studies with strains of pneumococci other than A66.1 were conducted by the same basic methods, except that the only dilution of the pre- or postimmune serum samples examined was 1:25.

The pneumococci used for challenge were prepared as aliquots and were stored frozen at −70°C in 20% glycerol [33]. Each aliquot was only thawed once. Any leftover bacteria were discarded. At least 2 aliquots of each lot were thawed to determine the numbers of colony-forming units per aliquot by growth on blood agar plates [34]. On each day that mice were to be infected, an aliquot of frozen pneumococci was thawed and diluted to the required concentration of pneumococci. The number of pneumococci injected was chosen to represent the lowest dose of pneumococci that could reproducibly kill all of the unprotected mice. A sample of each thawed aliquot was serially diluted and plated on blood agar plates, to ensure that the expected number of pneumococci were delivered. The numbers of injected colony-forming units recorded for each experiment were always based on the actual numbers observed rather than on the predicted number. The numbers of pneumococci injected on different days from different aliquots of the same lot never differed by >20%. All 9 separate experiments conducted in this study were carried out with the same aliquoted lot of A66.1.

To confirm our expectation that the observed fatalities among the infected mice were due to pneumococcal infection, we plated blood from 14 of the mice immediately after they died, following infection with A66.1. The blood was streaked undiluted on blood agar plates with and without 20 µg/mL optochin disks to aid in the identification of pneumococci [35]. The blood of all of these recently dead mice contained large numbers of pneumococci and lacked significant numbers of other bacteria. Mice that survived 21 days after intravenous injection of strain A66.1 were observed to be free of infection. All 24 randomly chosen mice infected with A66.1 but alive at day 21 showed no evidence of bacteremia. Identical results were obtained from examination of survivors and recently dead mice infected with the other strains of pneumococci.

**Results**

Pre- and postimmune serum samples from 5 subjects immunized with rPspA/Rx1 were examined to evaluate their ability to protect mice against challenge with the capsular type 3 strain A66.1. Control mice given Ringer’s injection solution before challenge died 1 or 2 days after challenge with A66.1, as expected [31]. Of the 9 preimmune serum samples examined, 4 (from subjects 52, 81, 90, and 114) showed evidence of at least low levels of protection. At a 1:25 dilution, these 4 preimmune serum samples were able to significantly extend the life of mice versus treatment with Ringer’s injection solution (table 3). However, none of the preimmune serum samples provided statistically significant survival, compared with Ringer’s solution treatment (table 3). To obtain evidence for the elicitation of protective antibody by immunization with rPspA, the protective capacity of the post- and preimmune serum samples from the 5 subjects immunized with PspA on alum were compared. Mice treated with 1:100 or 1:400 dilutions of the 5 postimmune serum samples lived significantly longer than did those treated with the preimmune serum samples (table 3). Two postimmune serum samples at the 1:1600 dilution even showed a significant increase, albeit small, in time to death of mice.

In terms of the fraction of mice that survived, the 5 PspA-immune serum samples all exhibited greater protection at a 1:100 dilution than did the preimmune serum samples from the same subjects. Two of the immune serum samples even caused a significant increase in survival at the 1:400 dilution (table 3). At a 1:25 dilution, all PspA postimmune serum samples showed statistically significant increases in either survival rates or time to death. The exception was for serum from subject 90: although the 1:25 dilution of postimmune serum was protective, the preimmune serum from this subject was also protective. Thus, statistically significant evidence for protection after immunization with PspA was apparent only at the higher serum dilutions, at which the preimmune serum had lost its protective efficacy.

It is expected that the increase in protective capacity of the serum samples after PspA immunization was due to the immune response to PspA. Support for this view, however, requires a comparison between the results obtained with PspA-immune serum samples and the serum samples obtained from subjects immunized with alum alone. All 5 postimmune serum samples from subjects immunized with PspA on alum became more protective, whereas all 4 serum samples from subjects immunized with alum alone did not become more protective after immunization. This association of PspA immunization with protection is significant ($P = .008$, Fisher’s exact test). There was a positive correlation between the amount of antibody to PspA administered to the mice and their chance of survival (figure 1). At a dose of 0.03–1.3 µg of IgG antibody to PspA, there was a log-linear relationship between the amount of antibody and the percentage of mice that survived (figure
Table 3. Effect of passive treatment with pre- or postimmune serum samples or Ringer’s injection solution on subsequent infections with *Streptococcus pneumoniae*.

<table>
<thead>
<tr>
<th>Passive treatment: immunogen, subject no. from which serum was obtained, immune status of serum</th>
<th>Anti-PspA, ( \mu g/mL )</th>
<th>Days to death, median</th>
<th>Survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1:25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1:100</td>
</tr>
<tr>
<td>rPspA + alun</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>Ringer’s</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>81</td>
<td>Ringer’s</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>87</td>
<td>Ringer’s</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>90</td>
<td>Ringer’s</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>115</td>
<td>Ringer’s</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Alum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Ringer’s</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>52</td>
<td>Ringer’s</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>84</td>
<td>Ringer’s</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>114</td>
<td>Ringer’s</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

NOTE. Pre- or postimmune serum at dilutions indicated or Ringer’s injection solution (0.1 mL) was given 1 h before intravenous challenge with 430–500 cfu of pneumococcal strain A66.1. Each group contained 10 mice. Immune serum samples were produced by immunizing subjects with recombinant pneumococcal surface protein A (rPspA). Mice were observed for 21 days. Survival time for mice alive at 21 days is reported as \( >21 \). In groups of mice in which exactly half of the mice died, a relevant median day of death could not be calculated. For such groups, the median day of death column lists the day of death of the last mouse to die followed by a comma and \( >21 \), the “time of death” of the remaining mice. Empty data cells indicate that no experiment was done under indicated conditions. \( P \) values for differences in time to death were calculated by 2-tailed Wilcoxon 2-sample rank test and for differences in survival were calculated by 2-tailed Fisher’s exact test. \( P \) values associated with preimmune serum samples indicate comparison between treatment with preimmune serum sample and Ringer’s injection solution; \( P \) values associated with postimmune serum samples represent comparison between treatment with pre- and postimmune serum samples. Pre, serum sample obtained before immunization; post, serum sample obtained 60 days after immunization.

<sup>a</sup> Data for mice that received Ringer’s injection solution are listed in this column.

<sup>b</sup> \( P < .0004 \).

<sup>c</sup> \( P < .002 \).

<sup>d</sup> \( P < .05 \).

<sup>e</sup> \( P < .01 \).

1). The 50% protective dose (PD<sub>50</sub>) was 0.2 \( \mu g/mouse \). To maximize the protective effect of the anti-PspA serum samples, 1.3 \( \mu g \) of antibody to PspA was required. Previous data indicate that IgG antibody given intraperitoneally equilibrates with the blood within 2 h [35]. Because mice have a blood volume of \( \sim 2 \) mL plus extravascular fluid, the protective concentration of antibody to PspA in their serum at the time of infection was probably <1 \( \mu g/mL \). Because the subjects were not immunized with capsular polysaccharide, it was assumed that their antipolysaccharide anti-
This suggests that the protection mediated by the preimmune levels of preimmune antibody to PspA or type 3 polysaccharide. The small degree of protection seen in a few of the preimmune serum samples was not completely explained by differences in the low antibody levels to type 3 polysaccharide therefore could not account for the increase in protection observed could have depended on the close antigenic similarities of the clade 2 immunizing PspA with the clade 2 PspA of the challenge strain. The challenge strain A66.1 expresses 2 PspAs; both are family 1, and one is clade 1 and the other clade 2 [20]. Thus, the protection observed could have depended on the close antigenic similarities of the clade 2 immunizing PspA with the clade 2 PspA of the challenge strain.

The ability of human antibody to PspA to exhibit cross-protection was examined by additional studies with serum sample 115. This immune serum sample could protect against challenge with A66.1 (table 1) and with each of 5 additional pneumococci bearing a spectrum of PspAs (figure 2). All 6 of these challenge strains expressed PspAs that were structurally and serologically distinguishable from the immunizing rPspA/Rx1 [13, 14, 20]. These 6 strains expressed 3 different capsular types (3, 6A, and 6B) and PspAs of both major PspA families 1 and 2 (table 2).

### Discussion

These studies were designed to determine whether immunization of humans with PspA could elicit protective antibody, as has been observed in numerous previous studies with animal serum samples [12, 36, 37]. As a surrogate for protection of humans, we have used a passive transfer mouse challenge model with highly virulent S. pneumoniae [38]. Our previous studies have shown that passive mouse and rabbit antibodies to PspA and active immunization with PspA can protect against infection of mice challenged by the intravenous or intraperitoneal routes [34, 37]. For the present studies, the intravenous route was chosen, because it results in less statistical variation in infection outcome and is expected to be more relevant to protection against bacteremia and sepsis than is intraperitoneal challenge. It is already known that immunization of mice with various rPspA/Rx1 constructs, including the one used in these studies, elicits protection against A66.1 and other pneumococcal strains [22, 24, 39, 40]. The rPspA/Rx1 immunogen used in the human subjects has been previously found to elicit protection in mice against pneumococci of PspA families 1 and 2 (authors’ unpublished data).

Solid evidence that human antibody to PspA could protect mice against fatal pneumococcal infection was provided by the observation that diluted preimmune human serum samples were poorly protective against pneumococcal infection, whereas diluted postimmune serum samples were strongly protective. This protection was clearly due to immunity to PspA, because no increase in protection was observed when mice were given human serum from subjects immunized with the alum adjuvant itself.

Our data from these passive protection studies demonstrate that the percentage of passively immunized mice surviving challenge can be related directly to the quantity of anti-PspA IgG in human serum. This relationship was observed, despite potential differences in antibody isotype, avidity, or fine specificity that may exist among the 5 selected postimmune serum samples. Therefore, these secondary parameters either are not influencing protection or are present in similar ratios among the 5 tested serum samples. Whichever is the case, it would be predicted that the same relationship between PspA IgG concentration and mouse protection exists for most other postimmune serum samples. Of the 25–29 subjects given 5, 25 or 125 µg of rPspA, 55%, 76%, and 100%, respectively, generated 4-fold increases in anti-PspA antibody levels, which suggests that most of the 83 subjects immunized with rPspA, for whom we had day 60 serum samples, generated substantial immune responses to PspA, regardless of the immunizing dose [13]. The 25 subjects immunized with the 125-µg dose of PspA increased their level of antibody to PspA by an average of 150 µg/mL. In none of them was the increase <10 µg/mL [13]. Our findings indicate that ~1.3 µg of human antibody was able to protect mice from fatal infection after intravenous challenge with pneumococci. This calculates out to be ~1 µg/mL of serum in the recipient mice. The lowest human anti-PspA antibody concentration on
day 60 was 2.15 μg/mL in a subject immunized with 5 μg of rPspA. These findings provide encouragement that rPspA immunization may elicit protective immune responses in humans.

It was of interest that the geometric mean levels of IgG antibody to PspA in preimmune serum samples were ~6 μg/mL, and 96% of the preimmune serum samples had ≥2 μg of antibody to PspA/mL. Studies are needed to determine whether the antibodies to PspA in normal serum samples, which have been elicited by natural exposure, are protective against infection in mice and humans.

Whether the same level of antibody to PspA is as protective in humans as it is in mice is not known. Moreover, people at highest risk of fatal pneumococcal infection may have deficits that might require especially high levels of antibody to protect them from most *S. pneumoniae* infections. In this regard, protein vaccines such as PspA may be particularly important. The high immunogenicity of these proteins and the ability of proteins to elicit anamnestic responses should make it possible to elicit high enough levels of protective antibody that offer protection to persons with the highest risk of infection.

The fact that all 5 of the subjects studied produced antibodies to PspA that were protective in mice was encouraging. It suggests that rPspA may be able to elicit a protective response, regardless of the immunological history of those immunized. Another encouraging finding was that the human antibody elicited to a single family 1 PspA protein, rPspA/Rx1, was able to protect against all 6 challenge strains, which expressed serologically and structurally different PspAs. Of these 6 strains, 4 were of sequence family 1, like Rx1, and 2 were of sequence family 2 [20]. Families 1 and 2 PspAs constitute ≥98% of pneumococci (authors’ unpublished data). Thus, it appears that even a single PspA may elicit antibodies that are protective against a large percentage of pneumococci.

The broad cross-protection observed with serum sample 115 was consistent with the broad cross-reactivity between individual human serum samples to rPspA/Rx1 and rPspAs of families 1 and 2 (table 4), as reported elsewhere [13]. Although it appears that much of the antibody elicited by the immunizing clade 2 PspA reacts with epitopes unique to that molecule, at least one-third of the antibodies that were elicited cross-reacted with ≥1 of the other PspAs [13]. Because the cross-reactivity of this 1 serum sample is very similar to the average cross-reactivity of the immune serum samples from which it was chosen [13], it would be anticipated that the broad protection seen with this serum sample also would be observed with anti-PspA serum samples from the other immunized subjects.

The 6 challenge strains used in these studies collectively expressed 3 different capsular types, 3, 6A, and 6B. All of the...
Table 4. Cross-reactivity of human serum samples with recombinant pneumococcal surface protein A (PspA) antigens representing different PspA clades and families.

<table>
<thead>
<tr>
<th>Clade, family</th>
<th>Serum sample from subject 115</th>
<th>Geometric mean antibody in 25 immune serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 1</td>
<td>28.7</td>
<td>23.6</td>
</tr>
<tr>
<td>2, 1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3, 2</td>
<td>38.7</td>
<td>27.6</td>
</tr>
<tr>
<td>4, 2</td>
<td>12.3</td>
<td>25.4</td>
</tr>
<tr>
<td>5, 2</td>
<td>16.5</td>
<td>14.0</td>
</tr>
<tr>
<td>6, 3</td>
<td>2.2</td>
<td>6.3</td>
</tr>
</tbody>
</table>

NOTE. All subjects were immunized twice with 125-μg doses of recombinant PspA. Micrograms of antibody cross-reacting with each of 6 prototype PspAs for serum sample from subject 115 and for each other immune serum samples from 25 subjects were calculated; next, geometric mean antibody levels of each of the immune serum samples were calculated. These values were used to calculate percentage cross-reactivity. Because the immunizing antigen was clade 2 (PspA/Rx1), the amount of antibody reacting with clade 2 was set to 100%, and cross-reactivity was calculated relative to clade 2 value. Information presented here was calculated from raw data [13].

challenge strains were highly mouse virulent and represented 2 different patterns of pneumococcal sepsis. The 4 type 3 strains cause a rapid sepsis and death, whereas deaths resulting from infections with type 6A and 6B strains usually do not occur for a few more days [31, 32]. The capsular diversity of pneumococcal challenge strains provided additional encouragement that immunity to PspA might be broadly protective in humans.

Although these studies examined only a small number of human serum samples, they confirm animal studies that indicated that, despite the diversity of PspA molecules, immunity to PspA is sufficiently cross-reactive to permit the use of PspA as a vaccine. Despite the cross-protection revealed in these studies, it would seem wise that any PspA-containing vaccine include ≥1 member of each of the 2 major PspA sequence/cross-reactivity families. PspA might be used as a vaccine by itself, as mixtures with other pneumococcal proteins [26, 41–43], or as a conjugate with polysaccharide antigens of pneumococci or other pathogenic bacteria [44]. As a carrier for pneumococcal capsular polysaccharide, PspA may be particularly appropriate. Immune responses to PspA–capsular polysaccharide should be able to be boosted better by pneumococcal infections than by conjugates of polysaccharide with nonpneumococcal surface proteins. Optimal cognate T cell help is elicited when T and B cell epitopes are presented to the immune system on the same macromolecular structure [45].

Acknowledgments

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