Antibody to Capsular Polysaccharide of Streptococcus pneumoniae at the Time of Hospital Admission for Pneumococcal Pneumonia

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IgG to capsular polysaccharide (CPS) of Streptococcus pneumoniae is thought to provide the greatest degree of protection against pneumococcal disease. Serum obtained at hospital admission from 14 (27%) of 51 patients with bacteremic pneumococcal pneumonia and 11 (37%) of 30 with nonbacteremic pneumococcal pneumonia contained IgG to CPS of the infecting serotype; these percentages are similar to the prevalence of IgG to CPS in a control population. However, when compared with antibody from healthy adults, this IgG had far less capacity to opsonize the infecting pneumococcal serotype for phagocytosis in vitro by normal human polymorphonuclear leukocytes or to protect mice against experimental challenge. Failure to opsonize correlated closely with failure to protect mice, and each of these parameters correlated well with poor avidity for CPS. Future vaccine studies may need to examine the functional capacity of antibodies as a surrogate for infection, in addition to measuring their concentration in serum.

We sought to determine whether, at the time of hospital admission, patients with pneumococcal pneumonia have antibody to the CPS of their infecting organism and, if they do, why this antibody is not protective. Our general method was to identify patients with pneumococcal pneumonia, retrieve the serum sample obtained from them at admission, serotype the infecting isolate, assay for antibody to the infecting CPS type by ELISA, and examine the functional activity in vitro and in vivo, if such antibody was present. We examined the in vitro activity by opsonization of an isolate of the same serotype for phagocytosis by human polymorphonuclear leukocytes (PMNL), and we examined the in vivo activity by the capacity to protect mice against challenge with this strain.

Methods

Subjects. Each day from 1 September 1996 through 31 March 1997 and from 1 September 1997 through 30 April 1999, one of the investigators reviewed bacterial culture results in the Microbiology Laboratory, Veterans Affairs Medical Center, Houston. Patients were initially considered for inclusion in this study if a culture of sputum or blood yielded S. pneumoniae. Patients were included (1) if they met case definitions (see below), (2) if ≥1 mL of serum that was obtained on the day of admission was available, and (3) if the infecting pneumococcal isolate was of a serotype contained in the 23-valent pneumococcal vaccine (the only types for which we regularly assay antibody). All sera were distributed to tubes in 0.5-mL aliquots. Sera and infecting bacterial isolates were stored at −70°C. Sera obtained on the day of admission are called “acute sera,” and sera obtained 10 days to 12 weeks after the onset of pneumonia are called convalescent sera.

Bacteremic pneumococcal pneumonia. Patients with the diagnosis of bacteremic pneumococcal pneumonia had a clinical pre-
sentation consistent with pneumonia (generally including ≥1 of the following: subjective fever, cough, sputum production, or pleuritic chest pain), radiographic confirmation of a pulmonary infiltrate, and ≥1 blood culture that yielded *S. pneumoniae*.

**Nonbacteremic pneumococcal pneumonia.** Criteria for diagnosing nonbacteremic pneumococcal pneumonia were stringent in order to exclude patients for whom the diagnosis might be questionable. Such patients had all or nearly all of the following: clinical presentation suggestive of pneumonia with cough, sputum production, subjective fever or chills (or both), physical findings of pneumonia, and a distinct infiltrate on plain chest radiography. Examination by microscope of a gram-stained sputum sample (re pneumococcal pneumonia, and a distinct infiltrate on plain chest radiography.

Culture was grown but who had no symptoms, signs, or radiographic changes suggestive of pneumococcal infection, and who showed no deterioration in the hospital that might have been consistent with an untreated bacterial disease. Such persons usually had a sputum sample cultured during evaluation of relatively stable lung lesions, such as pulmonary fibrosis, emphysema, or malignancy.

**Control sera.** Sera from 3 groups of healthy adults (control, positive control, and negative control subjects) were used individually or pooled. The control subjects were healthy middle-aged adults who had participated in studies of pneumococcal vaccination in our laboratory. Sera obtained from the participants before and 4 weeks after they received a 23-valent pneumococcal vaccine had been stored at −70°C. To determine the prevalence of IgG before and after vaccination, we assayed these sera for IgG to 5 common CPS (CPS 3, 4, 6B, 19F, and 23F). The positive reference serum pool was constituted by combining equal volumes of postvaccination sera from healthy control subjects. Positive control subjects were 5 persons selected from the above-mentioned 34 on the basis of having postvaccination IgG levels ≥5 μg/mL to the 5 serotypes under study. Negative control subjects were healthy adults who were chosen because pre- and postvaccination sera contained no detectable IgG to the 5 pneumococcal serotypes cited above [10].

**Serotyping.** All pneumococcal isolates were typed, using procedures that are described in detail elsewhere [11], with antisera obtained from Statens Seruminstitut (Copenhagen). Coagglutination with *Staphylococcus aureus* was used to amplify the effect of the antisera [11].

**ELISA for antibody to CPS.** Individual CPS (American Type Culture Collection, Manassas, VA) dissolved in PBS with 0.05% azide, 10–20 μg/mL, were incubated in wells of Immulon II plates (Dynatech, Chantilly, VA) for 5 h at 37°C and then incubated for 16 h at 4°C, as we have described elsewhere [12]. Before use, plates were blocked with PBS with 0.2% Tween 20 (PBST) for 1 h at 37°C and then washed 4 times with PBST. Sera were diluted 1:100 in PBS that contained 0.05% azide; isolated IgG suspensions from circulating immune complexes (CIC) were used undiluted. CWPS (10 μg/mL; Statens Seruminstitut) was added, and diluted sera and isolated IgG suspensions from CIC were incubated for 30 min at 4°C on a rocking platform, after which 2 3-fold dilutions were made from the already diluted, CWPS-adsorbed sera. Triplicate samples at each dilution were pipetted into wells coated with CPS and incubated at 37°C for 2 h, and wells were then washed 4 times with PBST.

IgG or CPS-reactive IgM was detected by the reaction of alkaline phosphatase–conjugated goat anti-human IgG or IgM and its sub-strate (Sigma, St. Louis). Each ELISA plate included 6 dilutions of a positive laboratory reference standard serum with a known concentration of IgG or IgM to a specific CPS based on the common reference serum 89-SF (provided by the Center for Biologics Evaluation and Research, Rockville, MD) as well as a negative laboratory reference serum that contained no IgG or IgM to the individual CPS under study [10]. Sera that yielded a result equal to or less than the negative reference serum were read as zero, although because of variation in the baseline and the asymptotic shape of the positive reference standard curve, a low level of IgG (generally <0.5 μg/mL) might, on occasion, appear to be present. Optical density was recorded by an automated reader (Dynatech).

After the antibody assays described in this study had been completed, we became aware of the possibility that adsorption with CPS from type 22F together with CWPS might remove cross-reacting IgG more completely than adsorption with CWPS alone. Sera that contained antibody were studied again after adsorption in this fashion; measured levels of IgG declined by ≤30%, and in no case was a serum that was regarded as containing antibody to the CPS of the admitting strain converted to one that seemed to lack antibody.

**Separation of immune complexes.** To precipitate CIC [13], we added 1 mL of serum to 1 mL of 12.5% polyethylene glycol in 0.1 M borate buffer (pH 8.4) and added 3 mL of distilled water to bring the volume to 5 mL. The resulting solution was maintained at 4°C for 90 min, after which the pellet was separated by centrifugation and washed three times with 2 mL of 2.5% polyethylene glycol in 0.1 M borate buffer. Antibody was dissociated by resuspending the precipitate in 1 mL of 0.1 M borate buffer (pH 10.4). IgG from the dissociated complexes was purified using 1-mL affinity columns (HiTrap protein A; Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer’s directions.

**Flow cytometry assay of phagocytosis.** Pneumococcal colonies were grown for 15 h at 37°C in 5% CO₂, on blood agar plates and then scraped from the surface of the plates and suspended in Hanks’ balanced salt solution without Ca²⁺ or Mg²⁺ (HBSS), yielding ~10⁸ cfu/mL. To count colony-forming units, we serially diluted and plated an aliquot. The suspension was washed twice, incubated at 56°C for 30 min to inactivate autolysin, washed again, and resuspended in 0.1 M NaHCO₃ buffer (pH 9). Fluorescein-isothiocyanate (FITC; 1 mg/mL; Sigma) was added, and the suspension was incubated on a shaking platform in the dark at 4°C for 18 h. FITC-labeled bacteria were washed 5 times and resuspended in HBSS to yield 10⁶ cfu/mL; these bacteria were dispensed to tubes wrapped in aluminum foil and stored at −20°C for use within 3–4 months.

PMNL were isolated from whole blood of healthy adults by sedimentation in Histopaque 1119 and 1077 (Sigma). Red blood cells were lysed by brief exposure to 0.2% saline, after which PMNL were
resuspended in HBSS with 0.1% gelatin (GHBSs) to 5 x 10⁶ cells/ml.

Bacteria were opsonized as follows. Each reaction mixture contained serum, which was diluted to yield 2 μg/mL IgG to the relevant serotype, 5 x 10⁶ cfu bacteria, and 6% complement-rich guinea pig serum in a final volume of 225 μL. If the IgG concentration of the original serum was below a level that can be diluted to 2 μg/mL in the final mixture, a lower concentration (but not ≧1 μg/mL) was used. This mix was incubated for 30 min in a vigorously shaking water bath at 37°C. The reaction was stopped by adding 1 mL of ice-cold HBSS. Opsonized bacteria were washed once and resuspended in 100 μL of GHBSs containing Ca⁺² and Mg⁺².

For the phagocytosis assay, flow cytometry was used to quantitate cell-associated bacteria by a modification of a previously reported method that included experiments showing that cell-associated bacteria were largely taken up by the phagocytic cells [14]. GHBSs (100 μL) with Ca⁺² and Mg⁺² and containing 5 x 10⁵ PMNLs were added to the opsonized bacteria to yield a ratio of 10 bacteria to 1 PMNL. The bacteria-PMNL suspension was incubated for 30 min in a vigorously shaking water bath at 37°C. The reaction was stopped with 1 mL of ice-cold GHBSs, followed by a wash, and the pellet was resuspended in 400 μL of GHBSs. Fluorescein from surface-attached bacteria was quenched by adding 100 μL of 0.1% trypan blue (pH 7.2) and incubating for 10 min at room temperature. The suspension was washed twice with 5 mL of GHBSs and resuspended in 350 μL of GHBSs with 1% paraformaldehyde (pH 7.35). Uptake of fluorescein-labeled pneumococci by PMNLs was measured by flow cytometry (FACSII; Becton Dickinson, Sparks, MD).

Duplicate samples of 10,000 cells each were analyzed. PMNLs were gated according to their characteristic light-scattering pattern. Log₁₀ fluorescein intensity (FI) was plotted against cell number. The FI generated by patient serum divided by the total FI generated was lighted for 12 h each day. Sera were diluted in saline to yield a range of concentrations of anticapsular IgG, and 0.1-mL volumes were injected intraperitoneally into groups of 4–5 animals [15]. For each experiment, groups of mice received diluted acute serum and, if available, convalescent serum from ≧1 patients and the positive reference serum pool with equivalent concentrations of anticapsular IgG. Control animals received 0.1 mL of saline. Absorption of particulate or dissolved material from the peritoneal cavity is rapid and complete [16]. Forty-five minutes later, mice were injected intraperitoneally with 10 LD₅₀ of the relevant pneumococcal serotype, which was obtained by thawing a well-characterized frozen stock and serially diluting it in saline. cfu were verified by dilution and plating after injection. Mice were observed daily for 7 days, after which no deaths occurred. The protective index was defined as the number of mice that survived after receiving the highest dose of IgG from a patient serum divided by the number of mice that survived at the same dose of IgG from the positive reference serum pool expressed as a percentage.

In phagocytosis and mouse protection experiments, a representative isolate of the same serotype as the infecting one was used to insure uniformity of data. In addition, because we have not been able to construct a column that successfully purifies anticapsular antibody, we did not use isolated IgG to CPS; rather, in each experiment, whole serum was adjusted by dilution to achieve the desired concentration of anti-CPS IgG. As a result, the possible contribution of other antibodies to phagocytosis in vitro and/or to protection of mice after pneumococcal challenge can not be excluded. However, in our assay, the absence of anticapsular IgG results in the failure to opsonize for phagocytosis, as shown above, or to protect mice, factors which seems to argue against an important protective role for other antibodies.

Determination of avidity constant by inhibition ELISA. Sera were diluted to yield 0.3 μg/mL IgG to the relevant CPS, a concentration which gives a reading of ≧1 optical density unit by ELISA. Diluted sera were incubated with CWPS to remove cross-reacting IgG. CPS was serially diluted to give concentrations ranging from 0.08 to 60 μg/mL and added to diluted sera; these mixtures were then incubated at 4°C on a rocking platform for 60 min. Diluted sera without CPS were studied to determine total free IgG. After this step, ELISA was done as described above except that serum incubation was limited to 1 h in order to minimize the disturbance of the solution-phase equilibrium, which may be caused by the binding of IgG to solid-phase CPS. Every ELISA plate included a laboratory standard with a known concentration of IgG to the relevant CPS.

IgG that was bound to CPS in solution was determined by subtracting free IgG, which was detected by ELISA (IgG bound to solid-phase CPS) from the total IgG as measured in sample without the inclusion of CPS. A Michaelis-Menten plot was constructed, with CPS (μg/mL) shown on the x-axis and bound IgG in solution (expressed in optical density units) on the y-axis. The association between anticapsular antibody and CPS substrate was determined by calculating the binding constant (Kₘ) and the saturation rate at which antibody binds CPS (Vₘax), according to the equation V = [Vₘₐₓ(S)]/(Kₘ + S), where V is the reaction rate and S is the concentration of CPS. Values of Kₘ and Vₘₐₓ were calculated by nonlinear regression data analysis (Graft; Erithacus Software,
Table 1. Presence of antcapsular IgG or circulating immune complexes (CIC) in serum from patients infected or colonized with Streptococcus pneumoniae.

<table>
<thead>
<tr>
<th>Complex, serum</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CPS IgG, acute</td>
<td>Bacteremic</td>
</tr>
<tr>
<td>Anti-CPS IgG, convalescent</td>
<td>Bacteremic</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacteremic</th>
<th>Nonbacteremic</th>
<th>Colonized</th>
</tr>
</thead>
<tbody>
<tr>
<td>14/51 (27)</td>
<td>11/30 (37)</td>
<td>8/19 (42)</td>
</tr>
<tr>
<td>8/13 (62)</td>
<td>9/13 (69)</td>
<td>2/4 (50)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. positive/no. studied (%). Acute serum, serum obtained at admission; convalescent serum, serum obtained 10 days to 12 weeks after the onset of pneumonia. CPS, capsular polysaccharide of the infecting serotype.

Staines, UK). The unit of $V_{max}$ was converted from optical density units to micromoles per milliliter per minute on the basis of the positive standard, the molecular mass of IgG (150,000 daltons), and the reaction time of 60 min. The avidity constant is defined as the ratio $V_{max}$ to $K_m$, reported as (μmol of bound IgG in solution)/μg of CPS/min.

Statistics. SAS software (version 6.12; SAS, Cary, NC) was used for $\chi^2$ and regression analyses. Analysis for continuous variables using Student’s $t$ test was performed with Excel software (version 5.0; Microsoft, Redmond, WA).

Results

Antibody to CPS. Serum from the day of admission (“acute serum”) was available from 100 patients: 51 with bacteremic pneumococcal pneumonia, 30 with nonbacteremic pneumococcal pneumonia, and 19 who were colonized with S. pneumoniae. Most patients lacked detectable IgG to CPS of the same type as their infecting organism. Accordingly, in subsequent experiments, opsonization was carried out with individual patient sera and the positive reference serum diluted to yield a final concentration of 2 μg/mL antcapsular IgG, with the exception of sera that contained <2 μg/mL of antcapsular IgG, as explained in Methods. Phagocytosis was studied at a ratio of 10 bacteria to 1 PMNL.

Results, reported as opsonic indices (defined in Methods), showed that serum antibody present at admission for pneumococcal pneumonia was poorly opsonic for the infecting serotype compared with that from colonized subjects, the positive reference serum pool, or individual positive control sera (study...
only for serotypes 4 and 23F). The geometric mean opsonic index for all acute sera studied was 16.7 for bacteremic and 13.4 for nonbacteremic pneumococcal pneumonia patients, compared with 58.0 for colonized subjects, 100 (by definition) for the positive reference serum pool, and 117 for positive control subjects (figure 2). The differences are significant for bacteremic or nonbacteremic acute sera versus positive control sera ($P < .005$) but not for colonized subjects ($P = .45$).

The finding that the geometric mean opsonic index for individual positive control sera closely resembled that of the positive reference pool serum validates the averaging effect that resulted from pooling sera. The geometric mean opsonic index was 6 for acute sera from 10 pneumonia patients who lacked antibody to their infecting serotype (serotypes 4 and 23F were used for these experiments), and the index was 14 for acute sera from 5 healthy adults (negative controls) who had no detectable IgG to CPS 4 and 23F. These indices were significantly lower than those for positive control subjects ($P < .001$; figure 2), and confirm that sera lacking antibody to CPS failed to opsonize for phagocytosis.

In convalescence, opsonization by serum from bacteremic patients remained low (geometric mean opsonic index, 21.3), while the geometric mean opsonic index from nonbacteremic patients rose to 118.2, a significant increase over that for acute sera from the same subjects ($P < .005$), and exceeded values for convalescent sera of bacteremic patients ($P = .12$). The geometric mean opsonic index in follow-up sera from colonized subjects rose to 84.8, approaching that for the positive reference serum pool (figure 2).

When results were stratified into low ($<50$) or normal ($\geq 50$) opsonic indices, acute sera from 11 of 12 bacteremic, 9 of 10 nonbacteremic patients, and 3 of 5 colonized subjects had low functional activity (table 2). Among the control subjects that we studied, 10 of 10 IgG-negative patients and 5 of 5 negative control subjects had minimal opsonic activity, whereas 10 of 10 individual positive control subjects, selected because they had IgG to CPS 4 and 23F, had normal opsonic activity. The differences in the proportions of persons whose sera had low or normal functional activity are significant when comparing positive control subjects and bacteremic or nonbacteremic patients or colonized subjects ($P < .05$ for each comparison). Antibody detected in convalescent sera was likely to be more functional, showing an opsonic index $\geq 50$ in 3 of 6 bacteremic, 7 of 8 nonbacteremic, and 2 of 2 colonized subjects (table 2). The increase in functional activity from 1 of 10 acute sera to 7 of 8 convalescent sera in nonbacteremic patients was significant ($P < .005$).

**Mouse protection.** The capacity of anticapsular antibody in patient sera to protect mice against challenge with mouse-virulent serotypes (types 3, 4, and 8 in this study) was then examined in vivo. In each experiment, patient sera and the positive reference serum pool were adjusted by dilution in saline to deliver a range of desired doses of IgG that varied with the serotype. In 2 separate experiments (figure 3), 11 ng of IgG to type 4 CPS from serum of a healthy adult nearly completely protected mice against challenge with *S. pneumoniae* type 4, and 33 ng of IgG was fully protective. In contrast, 33 ng of IgG from acute serum of a patient with type 4 bacteremic infection protected only 2 of 10 mice.

Figure 4 shows data from an experiment in which 150 ng of IgG to CPS type 3 from acute sera for 2 bacteremic patients with type 3 infection (the highest dose that could be administered in 0.1 mL of undiluted serum) provided no protection against type 3 challenge. In contrast, lower amounts of IgG from the positive reference serum pool or from a nonbacteremic patient’s acute or convalescent serum were partially protective. In this experiment, the positive reference serum pool was most protective, followed by the convalescent serum of the nonbacteremic patient and finally the acute serum of the same nonbacteremic patient. All experiments were repeated one or more times, limited in some instances by the amount of serum available. Similar results were obtained in other experiments. Statistical analyses were not performed on the protective index data because of the few data points for each category.

**Correlation of opsonic and protective indices.** Passive protection against lethal challenge correlated well with a high se-

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**Table 2.** Opsonic indices (OI; defined in Methods) of serum from patients with pneumococcal pneumonia and colonized subjects.

<table>
<thead>
<tr>
<th>Patients or subjects</th>
<th>Acute serum</th>
<th>Convalescent serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$\text{OI &lt;50}$</td>
</tr>
<tr>
<td>Bacteremic</td>
<td>12</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Nonbacteremic</td>
<td>10</td>
<td>9 (90)</td>
</tr>
<tr>
<td>Colonized</td>
<td>5</td>
<td>3 (60)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients. Acute serum, serum obtained at admission; convalescent serum, serum obtained 10 days to 12 weeks after the onset of pneumonia.
IgG to Capsule at Admission for Pneumonia

**Figure 3.** Protection of mice against challenge with *Streptococcus pneumoniae* type 4 by increasing admitting doses of anti-capsular type 4 IgG from the serum of a patient with type 4 bacteremic infection (○) or from serum of a healthy adult (●). Shown are cumulative results of 2 experiments.

**Figure 4.** Protection of mice against challenge with *Streptococcus pneumoniae* type 3 by increasing doses of anti-capsular type 3 IgG from serum of patients with type 3 infection at time of admission (open symbols) or in convalescence (closed symbols). ○ Bacteremic patient 1; □, bacteremic patient 2; △, acute nonbacteremic patient 1; ▲, convalescent nonbacteremic patient 1. * Positive reference serum pool. Bacteremic patients 1 and 2 did not survive, so convalescent sera (defined in Methods) were not available. In these experiments, there were 4 mice per group.

Discussion

Pneumococcal pneumonia remains a common and serious medical problem, especially among elderly persons and people with underlying diseases [1, 17–19]. Despite appropriate antibiotic therapy, mortality from bacteremic pneumococcal pneumonia in hospitalized patients approaches 25% [17–19], and there is fear that the rates of complications and death may increase with increasing resistance of *S. pneumoniae* to commonly available antibiotics. These factors have helped to rekindle interest in immunity to pneumococcal disease.

Throughout the past century, investigations of antipneumococcal immunity focused on antibody to CPS [1–3]; however, in the past decade, research efforts have broadened to include a possible role for antibody to other surface constituents as well [20–23]. Advances in methodology, first with the application of RIA [6] and later of ELISA [8, 9], made it possible
Of interest, the prevalence of antibody in our patients was about we found that about one-third of patients have such antibody. To be absent at the time pneumonia is diagnosed; in contrast, techniques were used in the preantibiotic era, antibody was said to the CPS of their infecting serotype. When less sensitive hospital, most patients with pneumococcal pneumonia lack of infection. We found that at the time of admission to the correctly the degree to which preexisting antibody to the CPS of pneumococcal challenge [4, 5].

directly the degree to which preexisting antibody to the CPS of an infecting organism might protect against the development of infection. We found that at the time of admission to the hospital, most patients with pneumococcal pneumonia lack antibody to the CPS of their infecting serotype. When less sensitive techniques were used in the preantibiotic era, antibody was said to be absent at the time pneumonia is diagnosed; in contrast, we found that about one-third of patients have such antibody. Of interest, the prevalence of antibody in our patients was about the same as it is in the population at large [12]. Of even greater interest was the finding that anti-CPS IgG present at admission had diminished functional activity. Unlike sera from healthy age-matched adults, acute sera from patients, when adjusted by dilution to yield a fixed amount of IgG to CPS, failed to opsonize the infecting pneumococcal serotype effectively in vitro in nearly every instance.

Most pneumococcal types are not virulent for mice (virulence defined as an LD of <10^5 cfu/mL). Using strains of S. pneumoniae types 3, 4, and 8 that were isolated from patients and are virulent for mice, we found that serum containing anti-CPS IgG failed to protect mice against challenge in vivo. Results of an in vitro test of avidity suggested that the nonprotective antibody fails to bind CPS avidly. Taken together, these results suggest that patients are prone to pneumococcal infection either because they lack IgG to CPS or because, if such antibody is present, it functions poorly.

In general, our patients exhibited increases in IgG to typespecific CPS after infection. The proportion of subjects with IgG to CPS of their infecting serotype increased from about one-third to about two-thirds, again similar to the proportion of normal subjects who developed antibody after pneumococcal vaccination. Antibody produced in response to infection was more likely to be functional, at least in patients who did not have bacteremia. Of the small sample of subjects whose convalescent serum was studied, 88% of nonbacteremic patients and 50% of bacteremic patients had functional antibody.

Generation of poorly functional antibody is not exclusive to older, diseased individuals. Nahm et al. [29] have shown that pneumococcal vaccination stimulates the appearance of IgG that is cross-reactive (i.e., reactive with related, nonvaccine serotypes) and that such cross-reactive IgG has less opsonic activity than homologous IgG. Usinger and Lucas [30] showed that because of the polymorphic nature of IgG, responses to CPS, healthy young adults who receive pneumococcal vaccine generate IgG with a range of avidity for the polysaccharide antigen. Several important problems remain to be addressed, including whether persons who generate poorly functional antibody to one polysaccharide do so to all polysaccharides, at what point and how (at a molecular level) the change from functional to nonfunctional antibody takes place, and whether presentation of antigen in a different form (e.g., as a protein conjugate) will stimulate production of better functioning antibody.

The results of the present study provide further insights into the nature of immunity to pneumococcal infection. In an early study, Musher et al. [7] reported a direct relation between the IgG level measured by ELISA and opsonization of pneumococci or protection of experimental animals. In addition, Vidarsson et al. [31] showed a high degree of correlation between the concentration of IgG to CPS and opsonizing capacity; the findings of both these reports were based on serum from healthy young adults. Usinger and Lucas [30] show good correlation.

Figure 5. Correlation of protective and opsonic indices (defined in Methods) is shown for all individual sera studied. Linear regression analysis revealed $r = .73$, $P < .001$. 

\[ r = .73; P < .001 \]

\[ \text{slope} = .88 \]
between avidity and opsonizing activity and between opsonizing activity and mouse protection. If serum from sick or elderly adults had been used in any of these studies, greater discrepancies might have been noted [26]. Of interest, IgG from patients with severe underlying disease was not as likely as that from normal subjects to opsonize pneumococci. It should now be clear why a protective level of IgG has not been determined and why antibody level can not serve as a surrogate for immunity: the degree of immunity may vary greatly, depending upon the functional activity of the antibody, and the population most in need of vaccine may generate antibody that is measured by ELISA but is poorly functional. Our findings underscore the importance of using functional assays, rather than simply measuring IgG levels by ELISA, for studying and comparing polysaccharide vaccines.

On the basis of the pilot study of Yee et al. [32], we studied patients with pneumococcal pneumonia, showing that those with bacteremic pneumonia have an increased risk of homozygosity for the R131 allele of the FCγRIIA surface receptor of PMNL [33]. R131 interacts poorly with Fc of IgG2, the IgG subclass that includes nearly all IgG to CPS, in contrast to the H131 allelic form, which avidly binds Fc of IgG2 [34]. Rodriguez et al. [35] found that cultured phagocytic cells transfected with the gene product that encodes H131 ingest opsonized pneumococci more readily than those transfected with the gene for R131. Accordingly, we sought, in the patients included in the present study, to determine whether there was an association between homozygosity for R131 and the presence of IgG to the infecting capsular serotype. Patients who had antibody to the infecting serotype were no more likely to be homozygous.
for the R131 allele than were patients who had no antibody. Only 2 bacteremic patients had functional antibody to the infecting serotype at admission, and the FCyRIIA genotype was determined in only 1 of them; this patient was homozygous for R131. Sanders et al. [36] showed that children with the R131 allele may also have reduced IgG; responses to pneumococcal vaccine, a finding of further interest in light of our previous and present studies.

Ten patients in this series had received 23-valent pneumococcal vaccine; IgG was detected in acute serum from only 1 of these patients. The absence of antibody in the remaining 9 patients might be consistent with genetic incapacity to respond to a polysaccharide [37], poor response due to age or underlying disease [4, 5], or rapid loss of antibody after an initial response, as suggested by Shapiro et al. [25]. Some researchers [2, 38] have proposed that pneumococcal vaccination protects against bacteremia but not against pneumonia. This concept does not seem to be supported by an understanding of the pathophysiology of the disease because, with inflammation, plasma pours into alveoli, providing a milieu that is not so different from the blood stream. By demonstrating similar rates of antibody presence and lack of function in bacteremic and nonbacteremic subjects, our study further opposes that hypothesis.

We investigated the possibility that anticapsular IgG is not detected in most sera because it is bound in CIC. Such complexes were identified in only 2 of 34 sera examined. Large amounts of CPS can be found in the serum of patients who are hospitalized with pneumococcal pneumonia, and the levels remain high for some time [39]; nevertheless, in most cases, anticapsular IgG also becomes detectable by 5–8 days after the onset of pneumonia and persists for months to years. Although patients with bacteremic or nonbacteremic pneumococcal pneumonia have been found to have elevated levels of CIC [39–41], it has not been shown that these complexes contain IgG to CPS of the infecting serotype. Our study did not exclude the possibility that IgG is bound to capsular material in tissues and does not spill into the circulation.

Acknowledgment

The authors are deeply indebted to the members of the Laboratory Service, Veterans Affairs Medical Center, Houston, without whose gracious cooperation this work could not have been done.

References


