Preimmunization Anti-Pneumococcal Antibody Levels Are Protective in a Majority of Patients With Cystic Fibrosis

Thomas Lahiri, MD, and David A. Waltz, MD

ABSTRACT. Objective. Although invasive pneumococcal disease is infrequent in cystic fibrosis (CF), it is recommended that all patients with CF receive pneumococcal immunization. As part of a comprehensive program to immunize our clinic population, we obtained preimmunization anti-pneumococcal antibody levels. We hypothesized that the percentage of CF patients without protective levels of anti-pneumococcal antibody levels would be high, as they are exposed to frequent antibiotic therapy that may eradicate organisms before generation of an antibody response.

Methods. An observational study of 100 patients with CF, aged 1 to 39 years, was conducted in a regional CF center. Preimmunization anti-pneumococcal antibody levels against 6 serotypes were measured by enzyme-linked immunosorbent assay. Protective antibody levels were defined as >200 ng/mL.

Results. A majority of CF patients—61% to 100%, depending on age and serotype—had protective levels of pneumococcal antibody. There was a significant positive correlation between antibody level and age for 5 of the 6 serotypes tested.

Conclusions. In contradistinction to our hypothesis, the majority of CF patients have protective preimmunization anti-pneumococcal antibody levels. However, a significant proportion—between 17% and 39%, depending on the serotype—did not exhibit adequate levels. Therefore, we concur with current recommendations for pneumococcal immunization in CF. Pediatrics 2001; 108(4). URL: http://www.pediatrics.org/cgi/content/full/108/4/e62; cystic fibrosis, Streptococcus pneumoniae, immunization, ELISA.

ABBREVIATIONS. CF, cystic fibrosis; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay.

Invasive disease associated with Streptococcus pneumoniae remains a serious problem in pediatric populations. Colonization of the respiratory tract by this organism is frequent and occurs in a majority of normal children.1-3 Individuals who lack the ability to clear encapsulated bacteria, such as those with sickle cell disease,4 splenectomy,5 and chronic renal disease,6 are at increased risk for the development of severe illness. In patients with cystic fibrosis (CF), S pneumoniae has been reported as the fourth most common bacterial organism isolated from sputa—after Pseudomonas aeruginosa, Staphylococcus aureus, and Haemophilus influenzae—and this prevalence may be underestimated, as S pneumoniae is difficult to isolate from sputa often heavily colonized with these other organisms.7,8 This pneumococcal colonization has been documented to be transient in CF patients even in the absence of antibiotic therapy.9,10

CF patients may have an attenuated antibody response to pneumococcus for the following reasons. Many CF patients have been treated, either prophylactically or intermittently, with oral and/or intravenous antibiotics, which usually cover S pneumoniae. This action may eradicate organisms before an antibody response can be generated.11 The abnormal airway secretions in CF and overgrowth of the respiratory epithelium by other organisms, such as P aeruginosa, also may impair the ability to recognize and mount an appropriate antibody response to pneumococcus. Indeed, Burns and May12 reported a decreased incidence of precipitating antibodies to pneumococcus in patients with CF compared with those with chronic bronchitis and control subjects. If true, this decrease in anti-pneumococcal antibodies may place CF patients at risk for severe illness. It has been recommended that all CF patients who are older than 2 years receive the 23-valent polysaccharide immunization against pneumococcus.3 With the hypothesis that a large proportion of patients would lack protective levels of anti-pneumococcal antibodies for the above-stated reasons and as part of a comprehensive effort to immunize our CF population, we evaluated preimmunization anti-pneumococcal antibody levels.

METHODS

From 1994 to 1997, we prospectively obtained antibody titers in adult and pediatric CF patients who presented to our clinic for routine ambulatory follow-up and who had no previous history of anti-pneumococcal immunization. Antibody levels to pneumococcal serotypes 3, 14, 19, 23, 26(6B), and 51(7F) were measured by enzyme-linked immunosorbent assay (ELISA).13 The serum from each patient initially was absorbed with S pneumoniae cell wall polysaccharide, which allows removal of antibodies that react with non-type-specific cell wall components of S pneumoniae. Samples then were added to a series of wells coated with the appropriate type-specific pneumococcal antigen (American Type Culture Collection, Manassas, VA). Quantitation was accomplished using US standard reference serum 89-SF2 (FDA, Bethesda, MD). This panel represents the 6 serotypes against which pneumococcal antibody titers are measured routinely at our institution. For serotypes 26 and 51, the Danish designation is given in parentheses.3 In addition to the pneumococcal serotypes, total serum IgG was measured in all patients.
A protective level of antibody was defined as ≥200 ng/mL. For each serotype, a linear regression analysis of the logarithmic transformation of anti-pneumococcal antibody level versus age was performed. A linear regression analysis also was performed for total serum IgG versus age.

This study was reviewed and approved by the Human Subjects Committee of Children’s Hospital.

RESULTS

Pneumococcal antibody levels were obtained from 101 consecutive CF patients who presented to our clinic for routine ambulatory follow-up. This number represented approximately 25% of our total clinic population. There were 43 male and 58 female patients with a median age of 13.2 years (range: 1–39 years). Fourteen patients were younger than 5 years; 1 patient was younger than 2 years.

A majority of CF patients were found to have protective levels of antibody against the 6 pneumococcal serotypes studied (Table 1). Of the 101 patients examined for antibody against serotype 3, 18 had levels below the protective level of 200 ng/mL. For serotypes 14, 19, 23, 26, and 51, the number of patients with antibody levels <200 ng/mL was 24, 11, 31, 15, and 16, respectively. The individual antibody levels of each patient for each serotype are depicted in Fig 1. As seen in Fig 1, the geometric means of the antibody levels varied between 456 and 870 ng/mL.

There was a positive correlation between total serum IgG levels and age (Fig 2). The number of patients with elevated IgG levels compared with our institution’s reference range increases over the second, third, and fourth decades, as was demonstrated previously. As shown in Fig 3, there also was a positive correlation between anti-pneumococcal levels and age for 5 of the 6 serotypes studied: serotypes 14, 19, 23, 26(6B), and 51(7F). Inspection of Fig 3 also reveals that anti-pneumococcal antibody levels below 200 ng/mL most were often seen in younger individuals.

DISCUSSION

Previous investigations have suggested a specific defect in the CF immune response. Although Abman et al did not detect hypogammaglobulinemia in any of the young CF patients diagnosed through newborn screening, the response to polysaccharide antigen has been questioned. CF patients were found to have a decreased IgG2 response to P aeruginosa lipopolysaccharide and lower levels of polyribosylribitol phosphate antibody (both total and IgG2 fraction) when compared with healthy control subjects. However, they maintained a normal level of antibody directed against protein antigen, such as tetanus. As CF patients may have an attenuated IgG2 response to encapsulated organisms and IgG2 subclass deficiency has been associated with increased susceptibility to pneumococcal disease in children, one might expect to see defective antibody responses to pneumococcus in CF patients. As stated previously, a blunted antibody response to pneumococcal colonization also may be attributed to chronic antibiotic and, in particular, anti-staphylococcal therapy. During the period of data collection, approx-

<table>
<thead>
<tr>
<th>TABLE 1. Percentage of CF Patients With Protective Levels of Antibody to Pneumococcal Serotypes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1–5</td>
</tr>
<tr>
<td>6–10</td>
</tr>
<tr>
<td>11–18</td>
</tr>
<tr>
<td>≥19</td>
</tr>
<tr>
<td>All</td>
</tr>
</tbody>
</table>

* Protective level is defined as >200 ng/mL.
approximately 90% of our CF patients were receiving such therapy (A. Colin, personal communication). For these reasons, we anticipated a blunted response to pneumococcus in our CF population.

Contrary to our original hypothesis, a majority of CF patients had protective levels of anti-pneumococcal antibodies against the 6 serotypes tested. This was true even for young patients. In fact, the percentage of patients with levels >200 ng/mL was similar to that in previously reported healthy control groups. As oropharyngeal colonization rather than lower airway colonization has been suggested to be the major impetus for pneumococcal antibody formation and as we did not examine the incidence of oropharyngeal colonization of \textit{S. pneumoniae} in our patients, it is possible that our patients had persistent oropharyngeal pneumococcal carriage and subsequent pneumococcal antibody production despite long-term antibiotic use.

Preimmunization anti-pneumococcal antibody levels were examined by other investigators in both chronically ill and healthy patients. These results are summarized and contrasted to those of the present study in Table 2. Baker et al.\textsuperscript{21} examined titers to serotype 14 in 44 healthy adults and found only 61% to be immune, using a protective level of 150 ng/mL

**TABLE 2.** Percentage of Patients With Protective Preimmunization Levels Against Serotypes 3 and 14 in Previous Studies and in the Present Study

<table>
<thead>
<tr>
<th>Study</th>
<th>Ages</th>
<th>n</th>
<th>% Immune (3)</th>
<th>% Immune (14)</th>
<th>Cutoff Value</th>
<th>Assay</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baker et al\textsuperscript{21} (1980)</td>
<td>24–37</td>
<td>44</td>
<td>N/A</td>
<td>61</td>
<td>150 ng/mL</td>
<td>RIA</td>
<td>Healthy adults</td>
</tr>
<tr>
<td>Mufson et al\textsuperscript{22} (1987)</td>
<td>Adult</td>
<td>21</td>
<td>67</td>
<td>100</td>
<td>500 ng/mL</td>
<td>RIA</td>
<td>Healthy adults</td>
</tr>
<tr>
<td>Musher et al\textsuperscript{23} (1993)</td>
<td>18–22</td>
<td>83</td>
<td>28</td>
<td>16</td>
<td>Reactive</td>
<td>ELISA</td>
<td>Military recruits</td>
</tr>
<tr>
<td>Musher et al\textsuperscript{23} (1993)</td>
<td>23–56</td>
<td>70</td>
<td>34</td>
<td>67</td>
<td>Reactive</td>
<td>ELISA</td>
<td>Healthy controls</td>
</tr>
<tr>
<td>Raby et al\textsuperscript{14} (1996)</td>
<td>2–17</td>
<td>19</td>
<td>53</td>
<td>37</td>
<td>200 ng/mL</td>
<td>ELISA</td>
<td>Healthy controls</td>
</tr>
<tr>
<td>Furth et al\textsuperscript{6} (1996)</td>
<td>2–21</td>
<td>41</td>
<td>59</td>
<td>32</td>
<td>150 ng/mL</td>
<td>ELISA</td>
<td>Chronic renal disease</td>
</tr>
<tr>
<td>Present study</td>
<td>1–18</td>
<td>62</td>
<td>76</td>
<td>69</td>
<td>200 ng/mL</td>
<td>ELISA</td>
<td>CF</td>
</tr>
<tr>
<td>Present study</td>
<td>19–39</td>
<td>39</td>
<td>92</td>
<td>87</td>
<td>200 ng/mL</td>
<td>ELISA</td>
<td>CF</td>
</tr>
<tr>
<td>Present study</td>
<td>1–39</td>
<td>101</td>
<td>83</td>
<td>76</td>
<td>200 ng/mL</td>
<td>ELISA</td>
<td>CF</td>
</tr>
</tbody>
</table>

Fig. 3. Pneumococcal antibody titers by serotype versus age in 101 CF patients. The solid lines represent the best fit by regression analysis. The dashed lines represent the 95% confidence intervals. A, Serotype 3 ($r^2 = 0.03$, $P = .08$). B, Serotype 14 ($r^2 = 0.13$, $P = .0003$). C, Serotype 19 ($r^2 = 0.08$, $P = .0042$). D, Serotype 23 ($r^2 = 0.04$, $P = .035$). E, Serotype 26(6B) ($r^2 = 0.10$, $P = .0015$). F, Serotype 51(7F) ($r^2 = 0.07$, $P = .0056$).
using radioimmunoassay (RIA). A significant proportion of healthy adults were found to be protected against serotypes 3 (67%) and 14 (100%) by Mufson et al\textsuperscript{22} again using RIA, but with a higher defined minimum protective level of 500 ng/mL. In 1993, Musher et al\textsuperscript{23} measured antibody titers by ELISA and found that as few as 16% of military recruits had reactive titers against serotype 14. They also demonstrated a low proportion of reactive titers to serotype 3 in both military recruits and other healthy adults.

Preimmunization anti-pneumococcal antibody levels were examined by Raby et al\textsuperscript{14} in 19 healthy pediatric patients. The geometric mean titers against serotypes 3 and 14 were 956 ng/mL and 211 ng/mL, respectively. In contrast, we found geometric mean titers of 565 ng/mL and 649 ng/mL, respectively, in CF patients of a similar age. Raby et al\textsuperscript{14} found that 53% and 37% had antibody levels >200 ng/mL for serotypes 3 and 14, respectively. Imposing the same age restrictions, our CF patients demonstrated a higher proportion of protective levels of antibody, 72% and 61% for serotypes 3 and 14, respectively. Furth et al\textsuperscript{1} investigated anti-pneumococcal antibody levels in children and young adults with chronic renal disease; 59% and 32% of these patients had levels >150 ng/mL by ELISA. Earlier studies on healthy children demonstrated a variable percentage of protected individuals before immunization.\textsuperscript{5,4,25}

Serotypes 3 and 14 have been examined frequently in many previous studies. Close examination of the response to serotypes 3 and 14, therefore, is justified. Type 3 is among the most immunogenic, even in children who are younger than 2 years.\textsuperscript{14,26–28} Type 14 has been associated with invasive disease in children, as well as with antibiotic resistance.\textsuperscript{29,30} The latter also has been studied in terms of its correlation with type III group B streptococcal antibody in mothers and infants.\textsuperscript{21} It is for these reasons that we chose to compare our results for serotypes 3 and 14 with those in the literature.

Several problems arise when attempting to interpret anti-pneumococcal antibody levels. The lack of a uniform standard for defining protective levels is an obvious barrier to interpretation.\textsuperscript{27,28} The level of antibody deemed to be protective for this study was 200 ng/mL. Various sources cite levels between 150 and 500 ng/mL as protective.\textsuperscript{14,19,28,31,32} Several reports have suggested that anti-pneumococcal titers >200 to 300 ng/mL afford the minimum amount of protection against bacteremia.\textsuperscript{24,25,33} These earlier studies used RIA to measure antibody levels. However, RIA overestimates anti-pneumococcal antibody levels because it cannot differentiate between capsular and cell wall polysaccharide.\textsuperscript{18} In addition, RIA cannot distinguish different immunoglobulin types, eg, IgG, IgA, and IgM.\textsuperscript{34} ELISA has the advantage of measuring only the IgG response. Unless antibody to IgM also is measured, ELISA may underestimate the total level of antibody.\textsuperscript{35} Nevertheless, ELISA has become the standard more recently\textsuperscript{35} and is the technique used in the present study.

Although pneumococcus has been recovered with regularity from CF sputa, its isolation is hampered by overgrowth with more aggressive organisms.\textsuperscript{7,8} Once \textit{S pneumoniae} grows from either respiratory secretions or blood, the subsequent determination of a particular serotype may be problematic. In addition, colonization of the respiratory tract in CF may be transient.\textsuperscript{9,10} For these reasons, it is difficult to correlate anti-pneumococcal antibody levels with the presence of this organism.

If, as our study suggests, the majority of CF patients have protective levels of anti-pneumococcal antibody, then the need for indiscriminate immunization becomes an issue. Exposure to additional polysaccharide antigen potentially could lead to a heightened inflammatory response. This, in turn, could result in additional lung damage, particularly in the presence of chronic colonization. Furthermore, as we measured antibody levels only in 1 child who was younger than 2 years, we cannot make any conclusions concerning the pneumococcal antibody response in infants and very young children with CF. However, we consider the proportion of patients who have nonprotective levels of antibody—7% to 39%, depending on the serotype—as significant enough to warrant immunization. Pending additional study, as the newer conjugated heptavalent vaccine is now available,\textsuperscript{36} we believe that this, too, should be given to the infant with CF.

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REFERENCES
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