Immune Response to Acute Otitis Media in Children

I. Serotypes Isolated and Serum and Middle Ear Fluid Antibody in Pneumococcal Otitis Media

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Seventy percent of pneumococci isolated from the middle-ear cavity of infants and children with acute otitis media were of one of the seven serotypes 1, 3, 6, 14, 18, 19, or 23. The immunological response in the serum and middle-ear fluid from otitis media caused by one of these serotypes was studied in 61 children by using either indirect hemagglutination or indirect fluorescent antibody tests, or both. Twenty-six of the patients had pneumococcal antibody present in the acute serum and 28 had it in the convalescent serum by at least one method. Thirteen of the 49 middle-ear fluids examined had antibody by the indirect fluorescent antibody technique. Serum pneumococcal antibody was found to reside predominantly in the immunoglobulin G or immunoglobulin M classes, whereas pneumococcal antibody with middle-ear fluid was found to be distributed equally among all three classes. Approximately 25% of the patients (16 of 61) had a positive immune response to their infection as evidenced by increased levels of pneumococcal antibody in the convalescent serum. The percentage of patients responding immunologically increased with age: 12% of infants less than 12 months showed a significant response, whereas 48% of children over 24 months responded.

The high incidence of acute otitis media in infants and children and the frequency of recurrent attacks make this otological disorder perhaps the most frustrating pediatric disease facing the clinician today. Despite successful antibiotic treatment of acute attacks, the rate of recurrence is high. The incidence of acute otitis media between birth and 10 years of age exceeds 75% (3, 10). Fifty-one percent have their initial episode in the first year of life, and almost half of these will have six or more episodes within the next 2 years (V. M. Howie, J. H. Ploussard, and J. L. Sloyer, Jr., manuscript in preparation). Recurrent otitis media can lead to persistent hearing loss with permanent impairment of verbal intelligence (6). In spite of the importance of this disease, little is known of how the body defends against it.

In a previous study we observed that approximately one-third of the episodes of acute otitis media was due to *Diplococcus pneumoniae* (8). We report here that 70% of these have been caused by the seven serotypes, 1, 3, 6, 14, 18, 19, and 23. We also report results of attempts to determine whether a specific immune response is evoked in the serum and middle-ear fluid (MEF) as a result of acute pneumococcal otitis media due to one of these seven serotypes.

MATERIALS AND METHODS

Patient population. All patients were seen by one of us in a private practice of pediatrics. The patients ranged in age from 1 month to 9.5 years; 50% were under 1 year of age. Patients were not observed to have clinical symptoms other than their otitis media and/or upper respiratory infection.

There were 61 patients analyzed, 53 whose acute and convalescent sera were studied by indirect fluorescent antibody technique (IFA) and 36 whose paired sera were studied by indirect hemagglutination (IHA); 28 of these 61 patients had sera assayed by both techniques.

Collection and storage of specimens. The techniques for collecting, culturing, and storing specimens have been described elsewhere (7, 9). All pneumococcal isolates were cultured on blood agar slants and sent to the University of Pennsylvania, where they were typed by the quellung reaction under the direction of Robert Austrian. Stock cultures were main-
RESULTS

Of 301 pneumococcal isolates which were serotyped, seven serotypes (1, 3, 6, 13) accounted for 70% of the episodes as summarized in Table 1. Eleven serotypes accounted for 86%, whereas an additional 20 serotypes accounted for only 14%.

The occurrence of pneumococcal antibody in acute and convalescent sera of patients with pneumococcal otitis media is summarized in Fig. 1. Both assays (IHA and IFA) showed similar results. Using results of either or both assays, approximately 42% of patients had pneumococcal antibody present in the acute serum and 46% had pneumococcal antibody in the convalescent serum. Almost half of the patients had antibody to the infecting pneumococcal serotype present in the serum at the time the infection was diagnosed.

The classes of pneumococcal antibody in the acute and convalescent sera are shown in Fig. 2. IgG pneumococcal antibody was present in 14 acute and 12 convalescent sera. IgM pneumococcal antibody was present in 11 acute and 6 convalescent sera, and IgA antibody in 4 and 6 sera, respectively. Three of the acute sera were from patients under 2 months of age, but only one of these had IgG pneumococcal antibody, which might have been of maternal origin.

A comparison of the pneumococcal antibody levels in the acute serum with the corresponding convalescent serum revealed that approximately 25% of the patients had a positive immune response by our definition, regardless of which of the two assays was used.

Twenty-five of 28 serum pairs analyzed by both assays showed identical results with re-

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**Table 1. Pneumococcal serotypes isolated from MEF of children with otitis media**

<table>
<thead>
<tr>
<th>Serotype isolated</th>
<th>No. of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14 (4.6)</td>
</tr>
<tr>
<td>3</td>
<td>21 (7.0)</td>
</tr>
<tr>
<td>4</td>
<td>12 (4.0)</td>
</tr>
<tr>
<td>6</td>
<td>29 (9.6)</td>
</tr>
<tr>
<td>7</td>
<td>12 (4.0)</td>
</tr>
<tr>
<td>11</td>
<td>11 (3.6)</td>
</tr>
<tr>
<td>14</td>
<td>28 (9.3)</td>
</tr>
<tr>
<td>18</td>
<td>15 (5.0)</td>
</tr>
<tr>
<td>19</td>
<td>63 (21.0)</td>
</tr>
<tr>
<td>22</td>
<td>12 (4.0)</td>
</tr>
<tr>
<td>23</td>
<td>41 (13.7)</td>
</tr>
<tr>
<td>Others*</td>
<td>43 (14.2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>301 (100.0)</strong></td>
</tr>
</tbody>
</table>

* Includes serotypes 5, 8, 9, 10, 12, 15, 16, 17, 20, 21, 27, 31, 33, 34, 35, 38, and 71, and three other serotypes for which uniparous sera were not available.
The MEF obtained when the acute serum was drawn was analyzed for the presence of antibody by using the IFA technique. Of the 49 patients whose acute sera and MEF were both suitable for analysis by IFA, 13 had pneumococcal antibody of at least one Ig class (IgG, M, or A) in the MEF, and 18 had pneumococcal antibody of some class in the acute serum. Table 3 summarizes the Ig class and the occurrence of pneumococcal antibody of the various Ig classes in these patients. Antibody in the acute serum was more often of the IgG or IgM class, whereas antibody in the MEF was distributed approximately equally in all three classes. It should be noted, however, that if antibody of the IgA class was present at all, it was more likely to be in the MEF. Since leakage of blood into the MEF occurred in some specimens, it was important to note the relationship between antibody in the bloody MEF and in the corresponding serum. Of nine instances of pneumococcal antibody both in the acute serum and MEF, six MEF were contaminated with blood, three of which had antibody of the same Ig class as the corresponding serum. Consequently, the source of the MEF antibody must be questioned in these specimens. In contrast, there were seven instances in which the acute sera contained pneumococcal antibody and the corresponding bloody MEF did not, suggesting that contamination of MEF with blood did not account for the antibody in the MEF and in agreement with previous studies suggesting that total Ig concentrations in the middle ear were not significantly affected by contaminating blood (9).

**DISCUSSION**

This study has revealed that 70% of the episodes of pneumococcal otitis media were due to only seven serotypes (serotypes 1, 3, 6, 14, 18, 19, and 23). Other data relating to the types of pneumococci causing otitis media in childhood have revealed that the principal serotypes in studies done several years ago were the same as ours (2, 5). Both the small number of serotypes involved in the majority of pneumococcal otitic infections and the relative consistency of the principal serotypes over the past 20 years demonstrate the feasibility of employing a pneumococcal vaccine containing several serotypes in the hope of preventing approximately three-fourths of pneumococcal otitis media.

Approximately half of the patients with pneumococcal otitis media had specific antibody directed against the infecting serotype present in the serum at the time the patient was

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**FIG. 1.** Occurrence of pneumococcal antibody in acute and convalescent sera as determined by IHA and IFA techniques. Numbers under each group represent the total number of serum pairs assayed by each method. The data indicate only that specific antibody is present in approximately 50% of both acute and convalescent sera. It is not a reflection of whether the antibody in the convalescent serum represents an increase over that of the respective acute serum as defined in the methods section. Such data appear in Table 2.

**FIG. 2.** Occurrence of each class of pneumococcal antibody present in acute and convalescent sera of 53 children, as determined by IFA.
In the determination of the existence whether this immune response is, antibody acute approximately if responders would be present by the initial infection. It is conceivable that some patients who had antibody in the acute serum had begun synthesizing antibody in response to their infection. This might explain the rather low percentage (25%) of those who were defined as positive responders. That is, true responders could include the approximately 50% of patients who had antibody in the acute serum as well as the additional 25% who developed antibody after the initial visit. Because no attempt was made to quantitate antibody by the IFA test, it is conceivable that some patients who were classified as negative responders would have been classified as positive responders if their sera had been titered. On the other hand, the correlation between the results of the IHA test and the IFA test (25 of 28) would suggest that few if any positive responders were missed by the IFA test. Furthermore, since the criterion for determining the status of the immune response by the IHA test is the classical one (fourfold or greater rise in titer) and because the correlation between the IFA and IHA tests was excellent, we suggest that the IFA definition for determining a positive immune response used in this study is valid.

Whether or not the antibody activity present in some acute sera represents a response to the current infection is unknown; however, at least some (25%) of the patients generated significant circulating antibody to the infecting strain after the onset of infection. Thus, it would appear that pneumococcal otitis media is capable of inducing humoral antibody to the infecting serotype.

Although it might be postulated that serum antibody could not be made available to the middle-ear mucosa in order to prevent the otitis, the apparent inefficacy of MEF antibody would still require explanation. The possibility that the concentration or quality of the antibody which we detected was not adequate to prevent the infection can not be excluded. It would be of considerable interest to quantitate the pneumococcal antibody by radioimmunoassay to determine the levels present in the MEF.

There was a direct relationship between age and the ability to mount a serological response to the infecting pneumococcal serotype. Similar observations have been reported for Haemophilus influenzae type b meningitis (13, 14), for vaccination with capsular polysaccharide from type b H. influenzae (15) or groups A or C meningococci (4, 12), and for streptococcal pneumonia (11). In each of these instances, antibody response was relatively poor until the second or third year of life. The reasons for the age-related differences remain to be elucidated. It is conceivable that neither the IHA nor IFA tests was sufficiently sensitive to detect small but significant changes in paired sera. Studies are currently under way to determine this possibility by using a more sensitive radioimmunoassay.

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LITERATURE CITED


