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Immune Response to Acute Otitis Media in Children

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

JOHN L. SLOYER, JR., VIRGIL M. HOWIE, JOHN H. PLOUSSARD, ARTHUR J. AMMAN, ROBERT AUSTRIAN, AND RICHARD B. JOHNSTON, JR.

Huntsville Hospital, Huntsville, Alabama 35801; Department of Pediatrics, University of California, San Francisco, California 94117; Department of Research Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104; and Departments of Pediatrics and Microbiology, University of Alabama Medical Center, Birmingham, Alabama 35801

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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-

Volume 9, no. 6, p. 1029, column 2, line 2: Change "... seven serotypes (1, 3, 6, 13)...." to read "... seven serotypes (1, 3, 6, 14, 18, 19, 23)...."

tained in blood broth and transferred every 2 weeks to fresh broth. Acute serum specimens and all MEF specimens were drawn at the initial visit, when the clinical diagnosis of otitis media was made. All serum and MEF specimens were stored at −20 C until analyzed. The mean time interval between acute and convalescent serum specimens was 15 days.

MEF was prepared for assay by gently but thoroughly suspending the specimens in two drops of phosphate-buffered saline (PBS). This suspension was diluted with four parts PBS, resuspended, and centrifuged to remove debris.

IFA test. Pneumococci grown on sheep blood agar were suspended in PBS, and one drop of the suspension was placed on a slide. One drop of FITC-conjugated antiserum to that serotype (Sylvania Corp., Milburn, N.J.) was placed on the dried smear and incubated for 5 min at 25 C. The slide was washed, dried, layered with glycerol containing 10% PBS (PBS-glycerol) and scanned in order to determine cell density and capsular fluorescence. The stock suspension was further diluted so that smears used in the IFA assay contained approximately 100 individually distinguishable organisms per oil-immersion (×1,000) field. One drop of either serum or MEF was placed on three smears of pneumococci, and the slides were incubated at 25 C in a moist chamber for 15 min. The slides were rinsed 10 times with cold water, dried on a warm plate, and placed in the moist chamber. FITC-conjugated goat anti-human immunoglobulin (Ig) G, IgM, or IgA (Hyland Laboratories, Costa Mesa, Calif.) was placed on one of the smears so that in each MEF or serum specimen the class of antibody present, if any, could be determined. The conjugated anti-immunoglobulin antisera were found to contain no inappropriate specificities by Charles Reimer, Center for Disease Control, Atlanta, Ga. The conjugates were incubated for 15 min at 25 C, washed and dried as before, layered with PBS-glycerol, and covered with a cover slip. Slides were coded and read by two individuals by using a Leitz microscope equipped with an HBQ 200-W mercury lamp, a BG-38 barrier filter, and a BG-12 exciter filter. There was a clear distinction between positive and negative capsular fluorescence and results could be recorded as either positive or negative. Controls consisted of human sera known to be positive and negative for antibody to specific pneumococcal serotypes and of FITC-conjugated anti-human Ig placed on the smears without prior serum.

A patient was considered to have a positive immune response if his convalescent serum had IgG or IgM pneumococcal antibody at a 1:5 dilution and his acute serum had no pneumococcal antibody at that dilution. A serum was considered to have antibody if positive fluorescence was due to any class (IgG, IgM, or IgA) at a 1:5 dilution.

IHA. IHA determinations were performed by Arthur Ammann, University of California School of Medicine, with chromic chloride-treated red cells by using the technique of Ammann and Pelger (1). A fourfold or greater rise in the titer of convalescent sera was considered a positive immune response. Any serum having a 1:2 or greater titer was considered to have pneumococcal antibody.

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 penumococcal antibody was present in 14 acute and 12 convalescent sera. IgM pneumococcal antibody was present in 11 acute and 16 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-

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

Serotype isolated	No. of isolates (%)
1	14 (4.6)
3	21 (7.0)
4	12 (4.0)
6	29 (9.6)
7	12 (4.0)
11	11 (3.6)
14	28 (9.3)
18	15 (5.0)
19	63 (21.0)
22	12 (4.0)
23	41 (13.7)
Others[a]	43 (14.2)
Total	301 (100.0)

[a] 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 unitypic sera were not available.

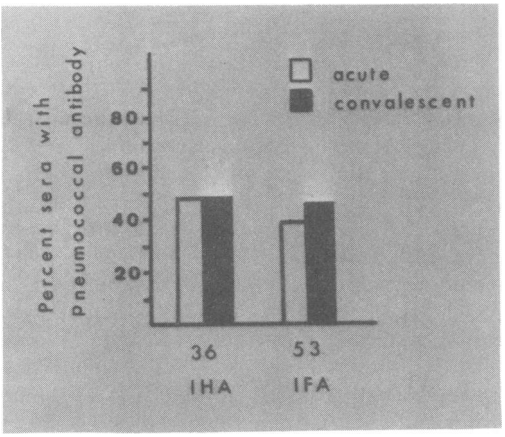

FIG. 1. *Occurrence of pneumococcal antibody in acute and convalescent sera as determined by IHA and IFA techniques. Numbers under each group represent the total number of serum pairs assayed by each method. The data indicate only that specific antibody is present in approximately 50% of both acute and 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.*

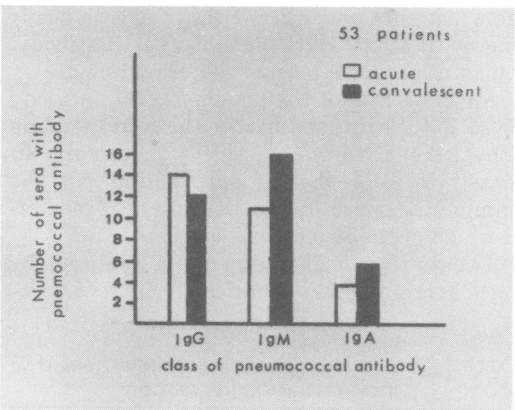

FIG. 2. *Occurrence of each class of pneumococcal antibody present in acute and convalescent sera of 53 children, as determined by IFA.*

spect to being considered a positive or negative immune response.

In an attempt to discover whether the age of the patient at the time of the episode of otitis media affected the immune response, the results with each assay were compiled according to the patient's age. From 10 to 13% of the patients under 12 months of age showed a serological response, depending on the assay used (Table 2). Between 28 and 43% of the patients between 1 and 2 years of age showed a response.

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 serums 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

TABLE 2. *Serum antibody response to the infecting pneumococcal serotype*

Method of assay	Response[a] at age (months):			Totals
	0-12	13-24	>24	
IHA	2/20 (10%)	3/7 (43%)	4/9 (44%)	9/36 (25%)
IFA	4/30 (13%)	2/7 (28%)	8/16 (50%)	14/53 (26%)

[a] The fractions represent the number of serum pairs showing an increase in titer divided by the total number of serum pairs assayed.

TABLE 3. *Class and occurrence of pneumococcal antibody in MEF and acute serums of 49 children with otitis media*

Pneumococcal antibody in	No. of occurrences of class		
	IgG	IgM	IgA
MEF only	4	4	5
Acute serum only	7	7	2
Simultaneous MEF and acute serum	5	3	1
Neither MEF nor serum	33	35	41

presented to the physician with his acute infection. In addition, approximately 25% had antibody in the MEF at this time. It can not be determined with certainty from our studies whether this antibody existed before the onset of the infection or whether it represents an immune response to that infection. Clearly, its existence did not prevent the otitis media.

Since we do not know the time interval between acquisition of the pneumococcus in the upper respiratory tract and the appearance of clinical otitis media, it would seem likely to us that many, if not all, 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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