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NOTES

Immune Response to Acute Otitis Media: Association Between Middle Ear Fluid Antibody and the Clearing of Clinical Infection

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Clearing of the middle ear fluid in patients with acute otitis media due to *Streptococcus pneumoniae* or *Haemophilus influenzae* was significantly associated with the presence and concentration of specific antibody in the middle ear fluid at the time of diagnosis.

Otitis media is the most common upper respiratory disease requiring antibiotic therapy. Despite intensive research efforts there remain major gaps in our knowledge of factors controlling the host's immune response to this disease (3, 4, 5, 7, 11). Specific antibody to the infecting organism has been demonstrated in middle ear fluid (MEF) of infants and children with acute otitis media due to *Streptococcus pneumoniae* or *Haemophilus influenzae* (9, 10). We report here the results of a study of the relationship of such antibody to clearing of the MEF by clinical criteria.

The study groups were the private patients of two of the authors. Only patients who returned to the clinic within 2 to 7 days (mean, 3 days) after the initial visit are reported in this study. Eighty patients under 3 years old are included. Ears were examined for MEF by using the pneumatic otoscope as described by Howie and Ploussard and scored as to clearance of fluid (2). Where MEF was deemed present at the follow-up visit, it was aspirated.

Antibody assays were performed on MEF obtained at the initial visit. Without exception, the follow-up visit 2 to 7 days later occurred before antibody analysis was done on the initial MEF. Laboratory personnel were unaware of the clinical status of the patient at the time antibody assays were performed. Only after the antibody assays were completed were the clinical records for each patient correlated with the antibody results.

MEF were cultured for bacteria according to standard methodology and frozen at −70°C (2, 3, 10). Pneumococcal isolates were serotyped by R. Austrian, University of Pennsylvania, by the Quellung reaction. *H. influenzae* were not typed, but each isolate was stored frozen in skim milk to preserve viability (2). The preparation of MEF for antibody determinations and the indirect fluorescent antibody test have been described (9). Briefly, MEF were thawed and one drop was diluted with four drops of phosphate-buffered saline. Specific antibody determinations were done by indirect fluorescent antibody using known pneumococcal serotypes or the patient's own strain of *H. influenzae*. Smears of bacteria were prepared and dried, and one drop of diluted MEF was added and incubated as described earlier (10). The presence and class of antibody was determined by using fluorescein-conjugated, heavy chain-specific goat anti-immunoglobulin G (IgG), anti-IgM, or anti-IgA (9, 10). Some MEF were examined for anti-pneumococcal capsular antibody by radioimmunoassay (G. Schiffman and R. Austrian, Fed. Proc. 30:658, 1971).

All but seven of the 80 patients included in this study were treated with either ampicillin, erythromycin-triple-sulfonamides, or penicillin-triple-sulfonamides. When analyzed by the chi-square method, no statistically significant relationship existed between MEF clearance and type of therapy ($P > 0.20$).

Of the 112 initial MEF obtained from the 80 patients, 40 MEF contained antibody of either IgG, IgM, or IgA class to the infecting bacterium and 72 MEF did not (Table 1). Twenty-three of the middle ear cavities which initially had antibody-positive MEF returned to the normal air-containing state by the next visit,

TABLE 1. *Relationship of specific antibody in MEF with clearance of the fluid*

Status of middle ear cavity	No. of MEF with:	
	Antibody present	Antibody absent
MEF cleared:		
S. pneumoniae otitis	6 (57.5%)	2 (12.5%)[a]
H. influenzae otitis	17	7
MEF not cleared:		
S. pneumoniae otitis	9	42
H. influenzae otitis	8	21
Total MEF	40	72

[a] $P < 0.0005$, by method of chi-square.

whereas only nine that initially contained antibody-negative MEF did so. This difference is highly statistically significant ($P < 0.0005$). Furthermore, when the etiology of the otitis media was considered and analyzed statistically by the method of chi-square, the association of MEF antibody with clearing remained significant (*S. pneumoniae* otitis, $P < 0.005$; *H. influenzae* otitis, $P < 0.005$). The clearing of MEF could not be correlated with the immunoglobulin class of antibody. In both the MEF that cleared and those that did not, either IgG or IgA antibody was found when antibody was present. In 8 MEF of the 23 that cleared, both classes of antibody were present. IgM antibody was not found in those MEF which cleared in the absence of one of the other classes. Of the antibody-positive MEF that did not clear, all except two had either IgG and/or IgA antibody. Those two MEF had exclusively IgM antibody. Because of the similar antibody class distribution in those MEF that cleared compared with those which did not, studies were done to determine antibody concentration to pneumococcal capsular polysaccharides.

Radioimmunoassay was used to determine whether differences in the concentration of MEF antibody to pneumococcal capsular polysaccharide could be correlated with clearing. The antibody nitrogen concentration of 15 MEF is summarized in Table 2. Seven MEF were cleared by the next visit. The mean antibody nitrogen/ml was 37.3 ng for MEF that cleared, whereas the mean concentration for fluids that persisted was only 8.1 ng of antibody nitrogen/ml. When analyzed by Student's t test, this difference is significant ($P = 0.03$).

The data suggest that specific antibody in MEF is significantly associated with returning the middle ear cavity to its normal air-containing state. Although several other studies have reported the occurrence of antibacterial antibody in body fluids (see for example references 1, 6, 8), we believe this to be the first observation that it may participate in clearing bacterial infection. Thus, association between specific antibody in MEF and a rapid clinical response to otitis media suggests a role for local antibody in resisting bacterial infections of the middle ear.

TABLE 2. *Relationship of concentration of pneumococcal capsular antibody in MEF with clearance of the fluid*

Concn (ng of AbN[a]/ml of specific antibody in individual MEF from 15 patients)	
MEF that cleared	MEF that persisted
0	0
19.2	0
21.6	0
22.4	0
44.2	9.6
76.8	12.0
76.8	17.6
	25.4
Mean 37.3	8.1

[a] AbN, Antibody nitrogen.

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