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Immunoglobulins of the Middle Ear Fluid in Acute Otitis Media: Relationship to Serum Immunoglobulin Concentrations and Bacterial Cultures

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Immunoglobulin concentrations were studied in 255 specimens of middle ear fluid (MEF) from 165 episodes of acute otitis media in children. There were significant amounts of all three major immunoglobulins (Ig) in MEF, the mean concentration of IgA being 39 mg/100 ml, of IgM 63 mg/100 ml, and of IgG 383 mg/100 ml. Secretory component was present in all 10 MEF specimens in which it was sought. In patients over 9 months of age, there was a decreased likelihood of isolating pathogenic bacteria from MEF if the patient had higher concentrations of IgA in MEF than in simultaneously obtained serum. IgA concentrations were greater in MEF than in serum in almost half the patients, and the mean MEF-serum ratio for IgA was 1.38. Thus, it would appear that in this disorder MEF represents primarily a secretory response to inflammation rather than a transudate.

The middle ear cavity normally contains air and is lined by both ciliated columnar and cuboidal respiratory mucosa (3). During otitis media the eustachian tube becomes occluded and the middle ear cavity fills with purulent fluid. The immunoglobulin (Ig) content of this middle ear fluid (MEF) has not been well characterized.

In this study we measured the level of IgA, IgM, and IgG in MEFs of 165 episodes of acute otitis media and compared these levels in some of the children to levels of serum immunoglobulins obtained at the same time. The data suggest that this fluid accumulation in the middle ear cavity represents, at least in part, a secretory immune response to infection of respiratory mucosa rather than a simple transudate.

MATERIALS AND METHODS

Patient population. All patients were seen with acute otitis media in a general practice of pediatrics between February and December, 1970. One-fourth of the patients were 2 weeks to 9 months of age, one-half were 10 to 24 months of age, and one-fourth were 25 months to 9 years of age. One-fourth of the patients were medically indigent. Eighty-six percent were white and 14% were black.

Collection and storage of specimens. MEF was collected in an Alden-Senturia apparatus, as previously described (5). MEF specimens (255) were obtained from 155 children with 165 different episodes of acute otitis media; 90 of these specimens were obtained during follow-up. After the removal of a loopful of MEF for culture, the balance of the MEF was capped tightly in the sterile collecting container and immediately frozen at −20 C until immunoglobulin determinations were performed.

Of the specimens obtained on initial visits, 87% were obtained by tympanocentesis and 13% were obtained from a ruptured ear drum. Of the follow-up MEF specimens, 64% were obtained without tympanocentesis. Specimens were cultured for bacteria on lamb blood and chocolate agar.

Immunoglobulin determinations. Serum specimens (205) drawn from the study patients at the initial or follow-up visit were stored at −20 C until analyzed. IgA, IgM, and IgG concentrations were determined by radial immunodiffusion (9), by using commercially prepared agar plates and immunoglobulin standards (Meloy Lab., Inc., Springfield, Va.). Sample (5 μliters) was delivered to each well. IgA determinations were made with antibody to serum IgA. Max Cooper, Univ. of Alabama Medical Center, determined the presence or absence of secretory piece in 10 MEF specimens by immunoprecipitation in agar gel (12), by using a monospecific goat anti-human secretory component donated by Richard Hong, Univ. of Wisconsin School of Medicine.

Statistical analysis. The numerical data were analyzed by using the Chi square test.

RESULTS

Serum and MEF immunoglobulins. MEF specimens were characterized as being obviously bloody or as having inconsequential bloodiness (no detectable bleeding or a faint pink tinge to the specimen). The ranges, means, and standard deviations of immunoglobulin levels of these two groups and of the total of all MEF specimens are given in Table 1. Approximately 62% of the specimens contained an inconsequential amount of blood. The difference in the means of immunoglobulin levels in the two groups was not significant, but there was a suggestion that the presence of blood diluted the MEF sample. Therefore, for the purposes of overall analysis (summarized in Tables 1 and 2), bloodiness was ignored; but for more careful analysis of MEF-serum immunoglobulin ratios (Table 3), only the values from the 64 specimens not containing frank bleeding were utilized.

Results from 165 episodes of acute otitis media which occurred in 155 patients are represented in Table 1. There were large amounts of all three immunoglobulins in the MEF. The usual age-related variations in serum immunoglobulin levels occurred in the children studied, but all of the values were within normal limits for age. There was not a significant relationship between mean levels of MEF immunoglobulins and the age, sex, race, or socioeconomic status of the patients, or the number of subsequent episodes of otitis media which occurred in these children.

Table 2 summarizes the immunoglobulin values from serum and MEF obtained on the initial visit from 118 different children with acute otitis media. The means of these 118 serum and MEF values do not differ significantly from the means of the total 255 MEFs or the total 205 sera analyzed. The mean value for MEF IgA approximates the mean value for serum IgA, but the mean values for IgM and IgG in the MEF are about half of the corresponding serum values.

Ratios of MEF to serum immunoglobulins. In individual patients, there was not a consistent relationship between MEF immunoglobulin levels and simultaneously obtained serum immunoglobulin levels. That is, a high (or low) concentration of any of the MEF immunoglobulins was not regularly associated with a high (or low) concentration of that immunoglobulin in the serum obtained immediately after myringotomy. To examine this relationship more closely, ratios of the simultaneously obtained MEF and serum immunoglobulin levels were established for the 64

TABLE 1. *Immunoglobulin levels in MEF and serum from all 165 episodes of acute otitis media*

Material analyzed	Immunoglobulin levels (mg/100 ml)									No. of specimens
	IgA			IgM			IgG			
	Range	Mean	SD[a]	Range	Mean	SD	Range	Mean	SD	
MEF, inconsequential bloodiness	4.0–250	44	36	2.4–500	67	64	14.5–2,700	417	310	158
MEF, bloody	2.5–170	31	28	8.8–360	56	49	68–1,000	325	192	97
All MEF specimens	2.5–250	39	33	2.4–500	63	59	14.5–2,700	383	271	255
All sera from above patients	0–160	41	38	13–330	129	73	95–1,450	663	293	205

[a] SD, One standard deviation.

TABLE 2. *Immunoglobulin levels in MEF and serum obtained simultaneously in 118 episodes of acute otitis media*

Material analyzed	Immunoglobulin levels (mg 100/ml)								
	IgA			IgM			IgG		
	Range	Mean	SD[a]	Range	Mean	SD	Range	Mean	SD
MEF	4.4–250	43	41	4.2–500	65	65	55–2,700	385	338
Serum	4.8–125	40	40	25–310	125	62	150–1,400	646	318

[a] SD, One standard deviation.

patients whose MEF specimen did not contain blood. These ratios are compared for all three immunoglobulins in Table 3. More patients had a MEF-serum ratio of >1 for IgA than for IgM or IgG. Of the 64 patients, 29 had a greater concentration of IgA in the MEF than in the corresponding serum, 11 had more MEF IgG than serum IgG, and 6 had a greater concentration of IgM in MEF than in serum. The mean MEF-serum ratio for IgA (1.38) was significantly higher ($P < 0.005$) than the mean ratio for IgG (0.71) or IgM (0.74), indicating a relative concentration of IgA in MEF.

To examine the possibility that greater MEF than serum levels of immunoglobulins might be due to loss of water from the MEF, MEF-serum ratios were compared for all three immunoglobulins in any of the 64 patients who had a ratio of >1 for at least one immunoglobulin. Of the 33 patients who had at least one ratio of >1, nine had such a ratio for two immunoglobulins and only two had such a ratio for all three immunoglobulins. Seventeen patients had elevation of only the IgA ratio; three patients had elevated IgM or IgG ratios alone without IgA elevation.

TABLE 3. *Distribution of the ratios of bloodless MEF-serum immunoglobulins in 64 patients*

Ratio ranges	No. of patients with MEF-serum ratio for		
	IgA	IgM	IgG
0.05–0.25	1	13	7
0.26–0.50	8	23	17
0.51–0.75	12	19	17
0.76–1.00	14	3	12
1.01–2.00	18	1	10
2.01–6.67	11	5	1

Since loss of water from the MEF would be expected to concentrate MEF immunoglobulins equally, water loss could not explain the selective concentration of IgA in the MEF.

In individual patients the MEF-serum ratios varied widely for the three immunoglobulin classes, only one patient having all three ratios in the same group of the arbitrarily grouped ranges listed in Table 3. Since transudation or simple dilution of MEF by serum leaked into the middle ear cavity would be expected to result in the same MEF-serum ratio for all three immunoglobulins, it would seem unlikely that the immunoglobulin concentrations found in the MEF resulted, at least entirely, from transudation.

Relationship between MEF-serum immunoglobulin ratios and organisms cultured from MEF. The interrelationship between the MEF-serum immunoglobulin ratios and the presence or absence of bacterial pathogens in cultures of the MEF was examined by comparing these variables for different age groups of patients. It was necessary to utilize all 118 MEF-serum pairs for this analysis to obtain sufficient numbers for significant comparison. Examination of the ratios for IgM and IgG failed to reveal any relationship to the culture results. However, examination of the ratios for IgA (Table 4) revealed a definite association ($\chi^2 = 12.0$; $P < 0.01$) between ratios of >1 (i.e., a concentration of IgA in MEF) and absence of the common pathogens (*Haemophilus influenzae*, *Streptococcus pyogenes*, and *Diplococcus pneumoniae*) in cultures from patients in the 10- to 18-month age group. Thus, 13 of 18 (or 72%) of those with ratios of >1 in the 10- to 18-month group showed no pathogens, in contrast to 8 of 18 (or 44%) in the younger group with ratios of >1. In addition, with increasing age there were fewer patients with concentration of IgA in the MEF ($\chi^2 = 12.5$; $P < 0.005$), as noted in Table 4.

TABLE 4. *Ratio of MEF-serum IgA related to bacteria cultured and age of patient in 118 patients with acute otitis media*

Results of MEF cultures	MEF-serum ratio for IgA					
	0–9 mo of age (30 patients)		10–18 mo of age (38 patients)		19 mo or older (50 patients)	
	≤1	>1	≤1	>1	≤1	>1
Bacterial pathogens isolated (*D. pneumoniae*, *H. influenzae*, *Streptococcus pyogenes*)	9	10 (56%)	14	5 (28%)	30	6 (55%)
No pathogens isolated	3	8 (44%)	6	13 (72%)	9	5 (45%)

DISCUSSION

The MEF of acute purulent otitis media has not been well described. The protein content (14, 17) and bactericidal activity (15) of the MEF in otitis media have been reported. Most investigations (14, 17) of the nature of MEF have been performed on fluid from patients with chronic, serous otitis media. Senturia classified MEF from serous otitis media on the basis of cell types found on smear, culture results, and total protein and polysaccharide content and has considered this fluid to be of inflammatory origin (14). Tonder and Gunderson reported that MEF from 10 patients with serous otitis media contained immunoglobulins A, M, and G, but that concentrations varied widely (17).

There has been considerable controversy in the past as to whether or not MEF represents an exudate or a transudate. Recent studies of MEF in serous otitis media have shown high concentrations of the enzymes alkaline and acid phosphatase, glutamic oxalacetic transaminase, and creatine phosphokinase in MEF (8), the presence of immunoglobulin-producing cells in the middle ear submucosa (7), and the presence by the immunoprecipitation (Ouchterlony) technique of immunoglobulins and secretory component in MEF (2). These findings led the authors to conclude that, in this disorder, MEF represents primarily a locally produced exudative response to inflammation. Our studies suggest that the MEF in acute otitis media is also an exudate, in that there was selective concentration in MEF of IgA (18), the principle secretory immunoglobulin of the respiratory tract, and that all 10 MEF specimens examined for secretory component contained this part of the secretory IgA molecule.

Approximately half of the 64 patients who had immunoglobulin determinations on simultaneously obtained MEF and serum had as much or more IgA in MEF than in serum, and the mean of MEF-serum IgA concentrations was 1.38. The radial immunodiffusion assay used to determine MEF IgA concentrations utilized serum IgA standards. Since the larger secretory IgA molecules present in MEF would diffuse more slowly into the agar than serum IgA (16), the actual concentration of IgA in MEF was undoubtedly even higher than reported here.

In addition to the high concentrations of MEF IgA, concentrations of IgM and IgG were also quite high in MEF. Immunoglobulin concentrations of this magnitude, especially for IgM, have not been found in secretions from other parts of the respiratory tract, for example, the nasopharynx and trachea (1, 4, 6, 11, 13). Interestingly, IgM was found to be the predominant immunoglobulin in lacrimal secretions from infants with trachoma of the eye (10). Although loss of water from the MEF might explain, in part, the high concentration of immunoglobulins there, neither water loss nor transudation of serum could explain the selective concentration of IgA in MEF.

A direct relationship was noted in the 10 months and older age group between concentration of IgA in MEF (MEF-serum ratio >1) and decreased isolation of bacterial pathogens. Moreover, those patients in the older age groups showing no concentration of IgA in MEF had an increased incidence of isolation of bacterial pathogens from the MEF. However, whether or not concentration of MEF IgA could be responsible for the decrease in the number of pathogens isolated remains to be elucidated.

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