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Secretory otitis media: Immunologic studies of middle ear secretions

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Thirty-one middle ear effusions obtained from children with typical serous otitis media were analyzed for immunoglobulin and secretory piece content. All specimens contained IgG and almost all contained IgA and IgM. IgE was found in 2 aspirates and secretory piece in 19. The secretory piece in middle ear effusions appeared to be immunologically identical with that present in other external secretions. It was also bound to IgA protein. IgG/IgA ratios of paired serum and secretions revealed considerable variations. Immunofluorescent studies of middle ear mucosa showed the presence of IgG, IgA, and IgM, and secretory piece. These studies indicate that the effusion in secretory otitis media is due at least in part to a locally active secretory process with local immunoglobulin production.

Secretory otitis media is a very common and important pediatric problem. It is the cause of a significant degree of acquired hearing loss and may be a precursor of permanent ear disease.

The etiology of secretory otitis is still unsettled. In particular the role of allergy has received conflicting support with widely varying opinions as to its importance. In order to examine this problem critically we have utilized several approaches. This report describes immunologic studies performed on middle ear secretions and tissue.

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MATERIALS AND METHODS

Middle ear effusion

Thirty-one middle ear effusions were obtained at the time of therapeutic myringotomy from 28 patients, aged 4 to 9, with typical serous otitis media. These effusions were obtained by suction with negative pressure and diluted in normal saline. The mucin and fibrin were removed by vigorous stirring with glass beads and centrifugation at 500 G for 30 minutes. The supernatants were dialyzed overnight against normal saline containing 0.001 per cent ethylenediaminetetraacetic acid (EDTA), passed through Millipore filters and stored at -20° C. until use. Serum was obtained from some patients at the same time.

Middle ear tissue

Mucosa biopsy specimens were obtained from the promontory areas of the middle ears from 3 patients with serous otitis media. Frozen sections of this tissue were prepared for immunofluorescent studies.

Antisera

Monospecific rabbit antisera to human IgG, A, and M and secretory piece and goat antiserum to human IgE were used for detection of immunoglobulins and secretory piece in middle ear effusions and sera.

Normal human IgG was isolated from serum by DEAE cellulose chromatography and then digested with papain. The digested product was fractionated by Sephadex G-200 gel filtration to remove undigested IgG. This was followed by starch block electrophoresis.¹ The Fc fragment thus obtained was used to immunize rabbits for production of monospecific antiserum.

Partially purified IgA and IgM, isolated from myeloma sera, were used to immunize rabbits. The antisera to IgA and IgM were rendered monospecific by absorption with human cord plasma which had been copolymerized with glutaraldehyde.

Monospecific goat antiserum to human IgE was obtained by a method previously described.²

Secretory piece was obtained by the method described by Tomasi and Bienenstock.³ The fat content and casein were removed from colostrum. The material was then fractionated with the use of starch block electrophoresis, DEAE and CM-52 cellulose chromatography, and Sephadex G-200 gel filtration to obtain purified IgA. Rabbits were immunized with this fraction. The rabbit antiserum was absorbed with human cord plasma to obtain antiseecretory IgA and absorbed with normal human serum which had been copolymerized with 2.5 per cent glutaraldehyde to obtain specific antiseecretory piece.

Detection and quantitation of immunoglobulins and secretory piece

Middle ear effusions were examined for IgG, IgA, IgM, and secretory piece content by double-diffusion gel precipitation with 0.9 per cent agarose gel in borate buffer.

For detection of IgE, ^{125} I-labeled anti-IgE was used instead of cold antiserum. This agarose plate was washed exhaustively with borate buffer and exposed to x-ray film. This technique is able to detect as little as 40 U. per milliliter of IgE.⁴

Quantitation of IgA and IgG was performed by the single radial immunodiffusion technique (Mancini). The IgG/IgA ratio of 10 paired secretions and sera was measured.

Immunoglobulin binding of secretory piece

Highly purified antibodies to IgG, IgA, and IgE were obtained by using immunoabsorbents. The IgG fractions of rabbit antisera to IgG and IgA and goat antiserum to IgE were obtained by DEAE cellulose chromatography with 0.01M phosphate buffer, pH 8.0, as the eluent. These IgG fractions were attached to immunoabsorbents of IgG, IgA, or IgE and eluted with 0.2M glycine HCl buffer, pH 2.3. Then, 3 mg. of this purified antibody was labeled with 1 mc. of ^{125} I using sodium iodide and chloramine T.⁵ Free iodine was removed by Sephadex G-25 and dialyses.

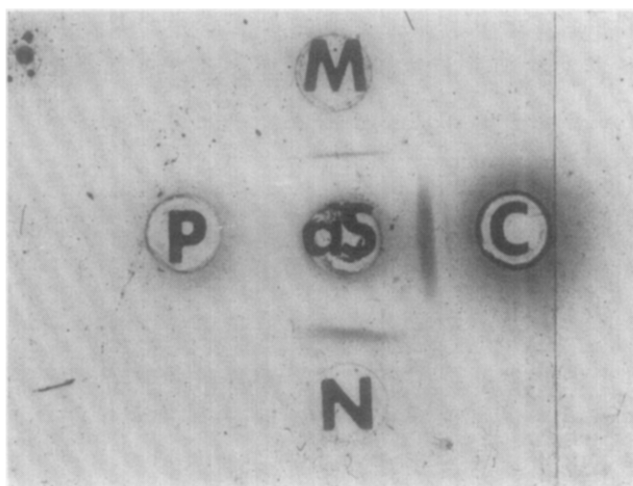


FIG. 1. Gel precipitation reaction of antisecretory piece rabbit serum (as) with middle ear secretions (M), colostrum (C), nasal secretions (N), and plasma (P).

TABLE I. Presence of immunoglobulins and secretory piece in 31 middle ear secretions

Immunoglobulin content				Secretory piece
IgG	IgA	IgM	IgE	
31	29	30	2	19

Radioimmunoassay was performed to detect binding of secretory piece by immunoglobulins. Middle ear effusion which contained secretory piece was reacted with antisecretory piece rabbit sera by agarose immunodiffusion. The plates were washed exhaustively with borate buffer. Thereafter the ^{125}I -labeled anti-IgA, anti-IgG, or anti-IgE was applied to a well and incubated for 24 hours in a moist chamber. These plates were washed exhaustively, stained, and then exposed to x-ray film. These experiments were designed to determine which immunoglobulin was bound to the secretory piece-antisecretory piece precipitin reaction.

Immunofluorescent studies

For immunofluorescent studies the rabbit and goat antisera were fractionated by DEAE cellulose chromatography and the IgG fraction obtained was then conjugated with fluorescein isothiocyanate (FITC). The free FITC was removed by filtration through Sephadex G-25. The conjugates were then passed through DEAE cellulose, eluted with 0.05M phosphate buffer at pH 6.3,⁸ concentrated and adjusted to $\frac{1}{4}$ U. for use.⁷ The F/P ratio of these conjugates varied from 1.3 to 1.8.

Frozen sections were fixed with cold acetone and stained with FITC-conjugated antisera. The specificity of staining was examined by a blocking test using cold antisera prior to the application of the conjugates.

RESULTS

Immunoglobulin and secretory piece content

All 31 middle ear secretions contained IgG and almost all had IgA and IgM (Table I). Two specimens contained IgE. Secretory piece was found in 19 specimens.

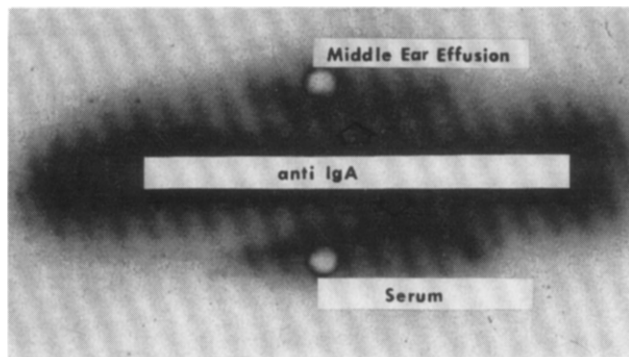


FIG. 2. Immunoelectrophoresis demonstrating the reaction of middle ear secretions (**top**) and serum (**bottom**) obtained from the same patient with anti 11S IgA rabbit serum. As the protein concentration in the secretion specimen was low, the precipitation reaction was difficult to visualize. In order to enhance visualization, ^{125}I -labeled anti-IgA was mixed with the cold anti-IgA in the center well. This figure shows the immunoelectrophoretic pattern as it appears in the developed x-ray film. As indicated by arrows, the electrophoretic mobility of IgA in secretions and serum is slightly different.

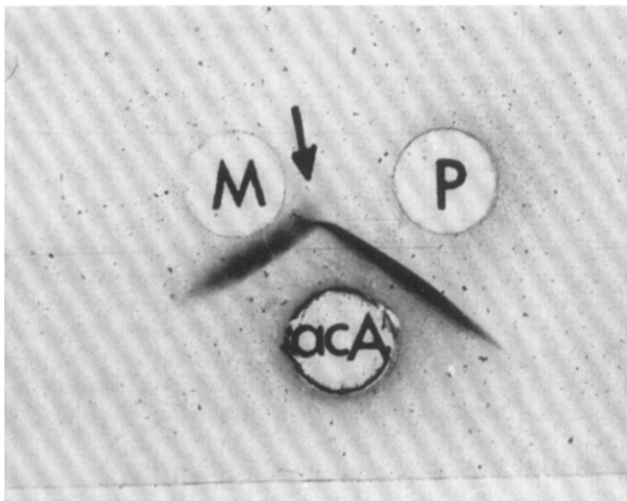


FIG. 3. Gel precipitation reaction of anti 11S secretory IgA rabbit serum (**acA**) with middle ear secretions (**M**) and plasma (**P**). The arrow indicates area of spur formation.

Studies of secretory piece

1. Antisecretory piece rabbit serum was reacted with middle ear secretions, nasal secretions, colostrum, and plasma. These results are shown in Fig. 1. A precipitation reaction was found with only the three external secretions, and a reaction of identity was suggested. No reaction with plasma was found.

2. The electrophoretic mobility of middle ear fluid IgA and serum IgA is slightly different (Fig. 2). The reaction between anti 11S IgA rabbit serum and plasma and middle ear secretions from one patient is shown in Fig. 3. A line

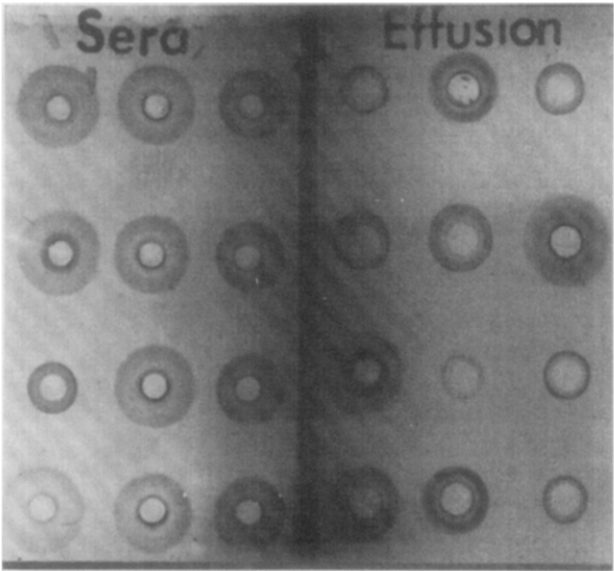


FIG. 4. Single radial immunodiffusion studies of the reactions of sera and middle ear secretions with anti 11S secretory IgA rabbit serum.

TABLE II. Comparison of IgG/IgA ratios in paired serum and secretions

Serum	Secretions
4.5	3.9
8.7	3.2
34.8	5.5
14.3	10.9
20.0	10.5
13.5	15.0
7.4	7.0
8.3	6.0
10.6	11.4
19.6	9.3

of identity was found with spur formation evident in the precipitin reaction between the middle ear secretions and antiserum.

3. Anti 11S secretory IgA rabbit serum was incorporated in agar and a group of serum and secretions examined by single radial immunodiffusion. Results are shown in Fig. 4. Single precipitin bands were found with sera indicative of the reaction between serum 7S IgA and antiserum. Secretions reacted with a double precipitin band. The outer band was interpreted as being due to 7S IgA-antibody reaction and the inner due to 11S IgA-antibody reaction.

4. The IgA/IgA ratio of paired serum and secretions from 10 patients was compared. In 4 of the 10 patients the IgG/IgA ratio in secretions was markedly lower than that of the serum (Table II).

5. Radioimmunodiffusion experiments revealed that secretory piece was specifically bound to IgA. No binding was present with IgG or IgE.

Examination of tissue

Histologic examination of these middle ear tissues revealed numerous polymorphonuclear cells and small round cells. Relatively small number of eosinophils and plasma cells were observed.

Immunofluorescent studies were performed using monospecific antiserum conjugated with fluorescein isothiocyanate. Immunoglobulins G, A, and M were readily detectable in all tissue. IgG was found diffusely in the interstitial tissue of the mucosa. IgA was contained in the epithelial and glandular cells. Plasma cells primarily contained IgA and IgG with little IgM. No IgE-containing cells were detected.

Positive staining for secretory piece was also apparent in the epithelium and apical portions of the glandular acini.

DISCUSSION

In the past, conflicting evidence has been presented in attempts to define the nature of the fluid in serous otitis media as either transudate or exudate.⁸⁻¹⁰ The results of the immunologic studies performed on middle ear secretions and tissue in this study indicate that the effusion in serous otitis media is at least in part due to a locally active secretory process with local immunoglobulin production. The extent of this active secretory process may vary among different individuals with serous otitis as well as in the same individual at different periods of time.

Secretory piece is a protein relatively unique to external secretions⁹ and rarely found in serum.¹¹ It is manufactured locally in tissue, becomes attached to IgA protein, and then is secreted into the external fluids. In these studies, secretory piece was identified in 19 of the 31 specimens examined. It appeared to be antigenetically identical with secretory piece found in other external secretions, colostrum, and nasal fluid. The secretory piece in middle ear secretions was bound to IgA protein, typical of its usual characteristics.

Further evidence for the active secretory bases of middle ear secretions came from analyses of the immunoglobulin content. There was extreme variation in the IgG/IgA ratios in the paired serum and middle ear secretions which were examined. In 4 of the 10 pairs, the ratio in the secretion was considerably lower than that of the matched serum. This suggests a relative increase in the IgA concentration of the secretory fluid as compared to serum and suggests that the immunoglobulins in secretions were not the result of simple transudation from serum.

Additional evidence for the exudative nature of these secretions is the recent finding by Tomasi of 2 antigens which are present in amniotic fluid and absent from plasma¹⁰ and now found in middle ear fluid.

Immunofluorescent studies of tissue obtained from the middle ear showed plasma cells containing IgG, IgA, and IgM. Secretory piece was readily detectable in epithelial cells as well as IgA. Previous histologic studies of middle ear mucosa have shown mucopolysaccharide at the apices of epithelial cells.¹⁰ These tissue studies further confirm the presence of an active secretory process in middle ear mucosa.

The role of the immunoglobulins in the middle ear secretions is not clear. Specific antibody activity of immunoglobulins in nasal fluid has been well described.^{12, 13} Whether similar activity is present in ear fluid is yet to be determined.

Of particular interest is the possible relationship of allergy to the etiology of serous otitis media. Two of the 31 secretions contained IgE. One of these came from an allergic child. Conversely, in this group of 28 patients there were 5 who were clinically atopic but lacked detectable IgE in secretions. We have subsequently found IgE in 3 other middle ear specimens, one of which came from an atopic child. Studies presently underway have also shown the presence of specific reaginic activity in several secretions. The significance of these latter observations is still speculative and not meant to imply an allergy etiology of secretory otitis media.

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