

Original article

*Evidence of eosinophil, neutrophil, and mast-cell mediators in the effusion of OME patients with and without atopy

Background: This study was designed to measure the involvement of eosinophils, neutrophils, and mast cells in the middle ear of patients with OME and to see whether that inflammatory response differed depending on whether or not the patient was atopic.

Methods: Levels of ECP, MPO, and tryptase were measured in effusion from 97 patients whose atopic status was determined by *in vitro* testing with 12 inhalants and five foods.

Results: Seventy-nine of 97 OME patients (81%) were atopic, among whom 86% (68/79) had elevated levels of effusion ECP, and 64% (23/36) had elevated tryptase. Mean ECP was 166 µg/l vs 3.38 µg/l, mean MPO was 6231 µg/l vs 116 µg/l, and mean tryptase was 4.8 µg/l vs 1.34 µg/l in atopic vs nonatopic ears. Total serum IgE did not differ between atopics and nonatopics ($P=0.28$).

Conclusions: The inflammatory response by eosinophils, neutrophils, and mast cells in the middle ear was distinctly different between atopic and nonatopic patients ($P<0.001$). This study provides evidence that eosinophils and mast cells, both essential to a Th-2 driven immune response, are present in the majority of ears with chronic OME, and supports the hypothesis that middle-ear mucosa, like that of the rest of the upper respiratory tract, is capable of an allergic response.

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Eosinophil, neutrophil, and mast-cell mediators were measured in middle-ear fluid from patients with otitis media with effusion (OME), and an attempt was made to determine whether the inflammatory response differed depending on whether or not the patient was atopic. Although children with hearing loss due to OME constitute potentially the largest group in the world with a reversible learning disorder, and several studies have suggested that OME is an immune-mediated disease (1), few otologists credit allergy with a direct role in the pathophysiology of middle-ear disease. However, the conventional approach to the treatment of OME has been unsuccessful at eradicating the disease. Meta-analysis has shown antibiotic therapy for chronic otitis media to be no better than placebo (2). It is time to consider a new clinical approach.

To understand the inflammatory processes that allow OME to persist, we must characterize the cellular constituents and their degree of activity in the diseased middle ear. The basic inflammatory response in the upper airway has been defined as either allergic,

involving a Th-2 response, or infectious, involving a Th-1 response (3). Conventional histology does not readily detect degranulated or activated eosinophils, neutrophils, or mast cells, and this has led to various conclusions. Mediators specific to the cells of inflammation that indicate active degranulation have now been identified, even though the cells themselves are often unidentifiable by light microscopy, as is the case with ear effusion.

Atopy involves a type I IgE-mediated hypersensitivity reaction in which activated mast cells and eosinophils participate in a Th-2 driven inflammatory reaction (4). Atopy has been defined as “the propensity of an individual to develop IgE antibodies”, and this condition is to be distinguished from that of an atopic patient with related symptoms who is deemed “allergic” (5). Past studies provide little support of a relation of allergy to otitis. Much of this may be a result of the poor sensitivity of the earlier methodology for determining atopy, which has been improved by *in vitro* testing (6).

The middle ear is essentially a fifth sinus which happens to harbor the organ of hearing. It is an extension of the upper respiratory tract, lined with pseudostratified ciliated columnar epithelium, and it is

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aerated via a narrow orifice, like the paranasal sinuses. We contend that the middle ear behaves like the rest of the respiratory tract, and that what has been learned about the atopic response in the sinuses and lungs may be applied to the ear to elucidate OME. For the middle ear to emulate the respiratory tract and as such be capable of an allergic response, it must be demonstrable that the cells and mediators necessary for the production of a Th-2 response are indeed present in the ears of patients with OME.

The purpose of this study was

- 1) to determine the degree of involvement of various inflammatory cells in middle ears with chronic effusion
- 2) to determine whether there was a difference in the inflammatory response in an OME ear depending on whether or not the patient was atopic.

Material and methods

Effusion subjects

To characterize the relation of allergy or infection to OME, we measured ECP, MPO, and tryptase in effusion from 97 individuals who presented with refractory OME to a solo, community-based otolaryngologist. Thirty-six children (age 14 months to 6 years), 41 children of school age (6–18 years), and 20 adults were selected in a consecutive, prospective manner. None was immunodeficient nor exhibited congenital malformations. All had documented hearing loss, flat tympanograms, and effusion of a minimum of 3 months' duration unresponsive to antibiotic and/or decongestant therapy. Middle-ear effusions (MEE) were collected at the time patients underwent routine myringotomy and placement of tympanostomy tubes (M&T). Allergy testing occurred after the patients were entered into the study to avoid preselection bias.

The effusion from 116 ears, including 19 pair, was collected quantitatively in a Juhn Tym-Tap® and diluted with precisely 2 ml of normal saline. Supernatants of diluted, centrifuged specimens were pipetted, stored at -20°C , and later measured for tryptase, MPO, and ECP. Variation of the volume of middle-ear fluid was previously considered by measuring 14 samples containing lithium chloride. Effusion volumes collected by our method were shown to be quite similar, ranging from 0.11 to 0.43 ml (mean $\pm$ 2 SD = 0.32 ml $\pm$ 0.11 ml) (7). That analysis demonstrated that any statistically significant differences observed between group means were unlikely to be explained by variation of volumes and dilution of MEE alone.

Some patients designated as having OME had also experienced a superimposed acute ear infection within 2 weeks before their M&T. These patients represent a mixed type of otitis (PUR-OME). Any patient with pus

in his/her effusion, or even a hyperemic tympanic membrane at the time of myringotomy, was included in this group. Ears that typified episodes of recurrent acute otitis media, which quickly resolve between infections, were excluded from the study. Among the 97 diseased patients were several children with no known antecedent infections who presented after failing a school hearing test. Typical patient histories and allergens have been described previously (7, 8). The results were sorted by patient type (i.e., atopic vs nonatopic), as well as by ECP, MPO, tryptase, total serum IgE, and age (Table 1).

In vitro testing

RAST or Micro-ELISA (Thabest-IgE™, Molecular Medicine, Inc., Denville, NJ, USA) testing of serum was performed for specific serum IgE in all patients to the same battery of 10 inhalant, two mold, and five food allergens (Der p 1 and Der f 1, cat, dog, timothy and meadow fescue grass, long and short ragweed, birch and oak trees, *Alternaria*, and *Hormodendrum* in addition to milk, egg, soy, corn, and wheat). Thabest-IgE values under 40 SIU may not be reliable in any quantitative sense, are considered nonreactive, and are regarded as negative for atopy. For the purpose of this study, the diagnosis was biased so that patients classified as atopic must demonstrate a positive *in vitro* test to at least two allergens. Atopy was determined without knowledge of the mediator results so as to have a singly blind study.

Table 1. Demographics and numbers of patients with various mediator levels in their effusion

	Nonatopic	Atopic	Total
No. of patients			
Child <6 years	3	33	36
Child 6–18 years	2	39	41
Total	5 (6.5%)	72 (93.5%)	77
Adult >18 years	13 (65%)	7 (35%)	20
Total	18 (19%)	79 (81%)	97
Age (mean)	44.7	9.2	
No. of patients with			
ECP			
>10 ($\mu\text{g/l}$)	2	68	70
<10 ($\mu\text{g/l}$)	16	11	27
Total	18	79	97
Fisher's exact test: $P < 0.0001$			
MPO			
<300 ($\mu\text{g/l}$)	13	4	
>300 ($\mu\text{g/l}$)	2	39	
Total	15	43	58
Tryptase			
<2 ($\mu\text{g/l}$)	7	13	20
>2 ($\mu\text{g/l}$)	0	23	23
Total	7	36	43

Titration of mediators

ECP, MPO, and tryptase in effusion were measured by double antibody radioimmunoassay (Pharmacia & Upjohn Diagnostic AB, Uppsala, Sweden), according to the instructions by the manufacturer (9). The interassay coefficient of variation for ECP was 3–8%; levels under 1 µg/l are undetectable. The MPO interassay coefficient of variation varied between 6 and 10%; levels under 8 µg/l were undetectable. The tryptase interassay showed a mean coefficient of variation of 5%; levels under 1 µg/l are undetectable.

The stability of ECP and MPO in middle-ear fluid was verified by incubating known amounts of ECP (2–200 µg/l) and MPO (8–1000 µg/l) in one of the ear samples for 1 h. This was then assayed for the two proteins. The recovery for ECP was $101.5\% \pm 5.3\%$ (SD) ($n=6$), and for MPO $102.9\% \pm 5.3\%$ (SD) ($n=7$) (10). Effusion mediator levels were considered to be abnormally elevated if ECP >10 µg/l (=control mean + 2 SD), if MPO >300 µg/l (=control mean + 2 SD) (7), or if tryptase >2 µg/l (=nonatopic mean + 2 SD). Initially, ECP levels were measured by RIA at a research facility in Sweden. In the final 2 years of the study, ECP, as well as all tryptase and RAST antibodies, was measured from 58 patients by the commercial UniCAP[®] System (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Statistical analysis

Statistical analyses were carried out by means of nonparametric tests. The Mann–Whitney U-test with Bonferroni's correction was used to compare the different groups (atopic *vs* nonatopic). Results are given as means $\pm$ SEM. The Spearman rank correlation coefficient was used to quantify the relationship between the ECP and tryptase, ECP and MPO, and MPO and tryptase in middle-ear effusion. Statistical calculations were performed by the statistical package, InStat[®] (GraphPad Software, San Diego, CA, USA), on a Power Mac 7200 personal computer.

Results

The demographic data on age distribution and atopic status are presented in Table 1. Evaluation of the type and number of antigens to which atopic patients had a positive RAST class 2 or greater revealed that, for inhalants, 42% of patients reacted to two to five antigens, and 39% had antibodies to six or more. In addition, 70% reacted at class 2 or greater RAST to both molds, 6% reacted to just one, and 51% had significant reactions to foods. Thirty-seven percent demonstrated antibodies to one or two foods and 14% to three to five foods. Retrospective review of the charts, including written inquiry to the referring physician, revealed that 62% had documentation of

additional atopic signs and symptoms including asthma (22%), allergic rhinitis (48%), eczema (4%), and chronic nasal congestion (8%).

There was insufficient fluid to measure all mediators, so the total number of patients tested for each mediator varied. Although below detection level of the assay, tryptase values below 1 µg/l were recorded as raw values for the purpose of graphs and statistical means.

Atopic status

Eighty-one percent of 97 OME patients (79/97) were atopic by *in vitro* testing. Among the 20 adults, the prevalence of atopy at 35% was higher than that found in the general population. Among the children, 72/77 (93%) were atopic (Table 1). Eighty-nine of the 97 patients were classified as having refractory, nonacute OME, and eight patients had signs of a recent infection (PUR-OME).

Mediator levels in effusions

Table 2 lists mean values for each mediator among ears from atopic and nonatopic patients. The inflammatory response by eosinophils, neutrophils, and mast cells in the middle ear (Figs. 1–3) was distinctly different depending on the patient's atopic status ($P<0.001$). ECP was elevated (>10 µg/l) in 68/79 (86.1%) atopic patients (mean 165.8 µg/l).

Tryptase was elevated (mean 4.8 µg/l) in the effusion from 23/36 atopic patients (64%). Tryptase was below

Table 2. Mean mediator levels in 116 middle-ear effusions and total serum IgE from 97 patients with OME

	Nonatopic	Atopic	Total
Effusion ECP			
No. of ears	21	95	116
Mean	3.38	165.82	
SD	3.5	240.26	
$\pm$ SEM	0.76	24.65	
$P<0.0001$			
Effusion MPO			
No. of ears	18	51	69
Mean	115.96	6231	
SD	125.32	8018	
$\pm$ SEM	29.54	1122	
$P<0.0001$			
Effusion tryptase			
No. of ears	8	49	57
Mean	1.34	4.78	
SD	0.39	5.09	
$\pm$ SEM	0.14	0.73	
$P=0.009$			
Total serum IgE			
No. of patients	18	76	94
Mean	42.29	93.8	
SD	55.7	157.8	
$\pm$ SEM	13.13	18.1	
$P=0.28=NS$			

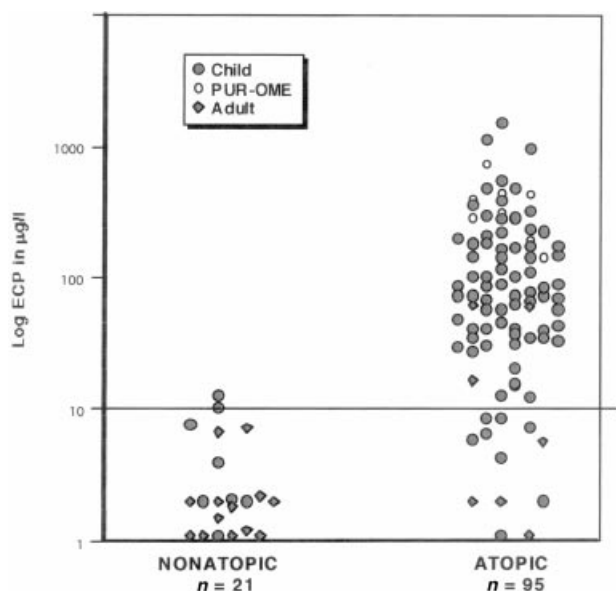


Figure 1. ECP in 116 ear effusions.

2 µg/l in all seven nonatopic patients as well as in one PUR-OME and 12 atopic patients (Fig. 3). There was no correlation of tryptase to either MPO or ECP (Spearman, $P > 0.05$).

The highest levels of MPO were found in ears which had a superimposed infection at the time of myringotomy (PUR-OME). Neutrophils were significantly active in all atopic ears, producing mean MPO levels 53 times higher than that measured in nonatopics (Fig. 2, Table 2). Pus in the middle ear produced the highest eosinophil and neutrophil mediator levels, but had no apparent effect on mast-cell involvement, although the sample size was small.

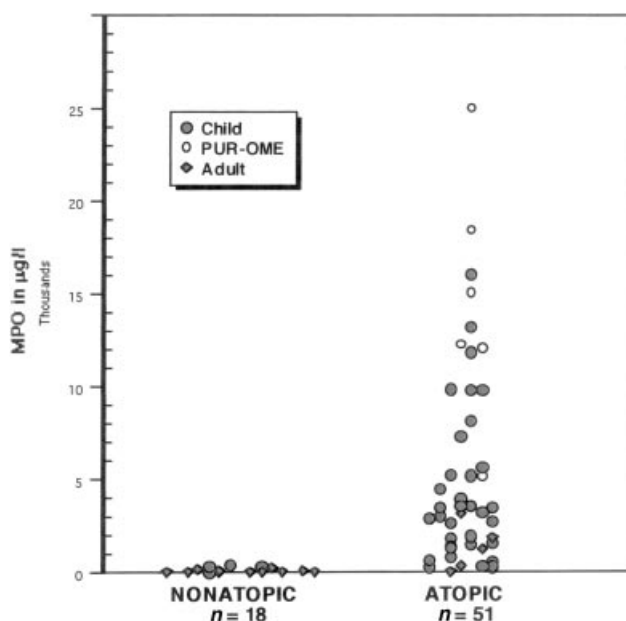


Figure 2. MPO in 69 ear effusions.

Most atopics had serum IgE of < 100 µg/l (Fig. 4). Total IgE did not differ between atopics (mean 93.8 µg/l) and nonatopics (mean 42.2 µg/l) ($P = 0.28$) (Table 2).

Discussion

To our knowledge, this study is the first to correlate the expression of markers of eosinophil, neutrophil, and mast-cell activity found in middle-ear effusion from patients with OME with their atopic status as characterized by *in vitro* testing.

One of the pitfalls of any study is the method used to diagnosis atopy. Conventional skin testing methods are subjective, highly variable, and influenced by a variety of mechanisms including patient skin response and tester inconsistency. Poor standardization of both antigen and testing techniques has been shown to lead to a discrepancy between skin test and *in vitro* IgE antibody results of more than 100-fold (6). Although the skin prick test offers high specificity, its sensitivity is less than 45% (11), making it less reliable than a coin toss in diagnosing atopy, especially in disease involving only mild or moderate elevation of IgE. We chose *in vitro* testing for our study. Its sensitivity of 90% makes it likely that most atopic patients were identified, with a minimal number of false negatives (6).

Correlation of atopy and mediators

The majority of atopic OME patients, unlike the nonatopics, demonstrated significant involvement of eosinophils and mast cells, as reflected by increased levels of both ECP and tryptase in their middle ear ($P < 0.001$) (Table 2). ECP was elevated (> 10 µg/l) among 68/79 (86.1%) atopics (Fig. 1). Elevated ECP correlated to a patient's being atopic (Fisher, $P = 0.001$) and was found to serve as a marker of atopy among patients with nonpurulent effusion with a positive predictive value of 97.1% and a diagnostic sensitivity of 86% (Table 1). Tryptase was > 2 µg/l in 23/36 (64%) atopic patients. Cell kinetics would suggest that these elevated levels of ECP and tryptase reflect an active inflammatory process (12). Biopsies of middle-ear mucosa from atopic patients have shown active degranulation of both eosinophils (13) and mast cells (14), and correlate with the elevated levels of the respective effusion mediators.

Tryptase was < 2 µg/l in 13/36 (36%) atopic patients. ECP was below significance in 10 atopics. This suggests that elevated mediators reflect a local activation of eosinophils and mast cells, not a general systemic response to being atopic. The dilution of the effusion specimens in this study is an important consideration. If we assume an average volume of 0.3 ml of effusion diluted during collection with 2 ml of saline in order to wash the thick mucoid samples from the 20 French

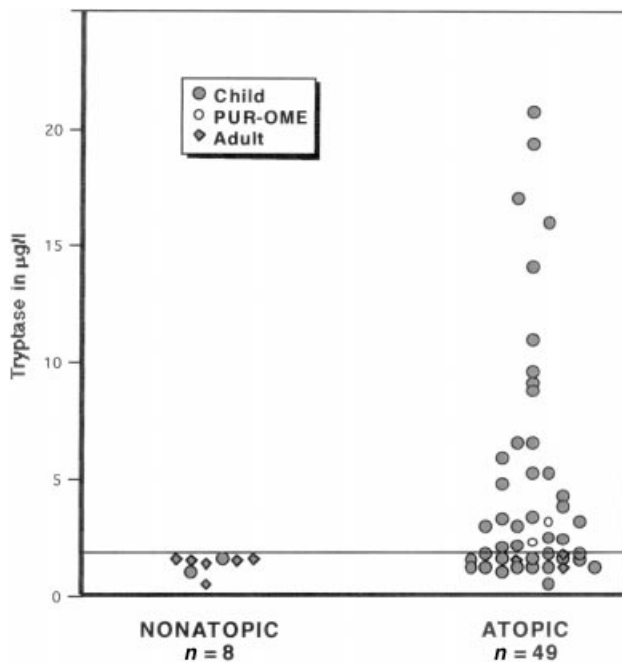


Figure 3. Tryptase in 57 ear effusions.

suction tubes (7), the absolute mediator concentrations in OME ears was actually six to seven times greater than that recorded in Table 2.

Correlation does not prove causality. Other reasons that as many as 20% of atopic patients had low levels of ECP or tryptase may be that a patient's atopy was unrelated to his/her ear disease, or that the atopic response may have been at a stage of resolution, or that, due to the dilution of tryptase, the sensitivity of the current assay may be too low.

Allergy vs infection

Purulence in the middle ear has previously been shown to elevate both eosinophil and neutrophil mediators (7). In this study, gross pus had a similar effect on ECP and MPO (Figs. 1 and 2) but did not appear to have an effect on mast-cell activity (Fig. 3). Most atopic patients had increased levels of MPO in addition to ECP in their ear effusion, whether or not they showed evidence of acute inflammation. Paired samples confirmed that in an acutely infected ear purulent OM was associated with a significant elevation of both mediators, but with a disproportionately greater elevation of MPO, as reported earlier (7).

The contribution of neutrophils to middle-ear disease is unclear. It was expected that neutrophils would be present in purulent OME (15). We were surprised to find that neutrophils were so very active in nonacute OME. The late asthmatic response in animal models is dependent on neutrophil availability and neutrophil chemotactic factor. This factor has been demonstrated in both atopic and nonatopic subjects (16). Neutrophils from atopics produce more superoxide in the absence of

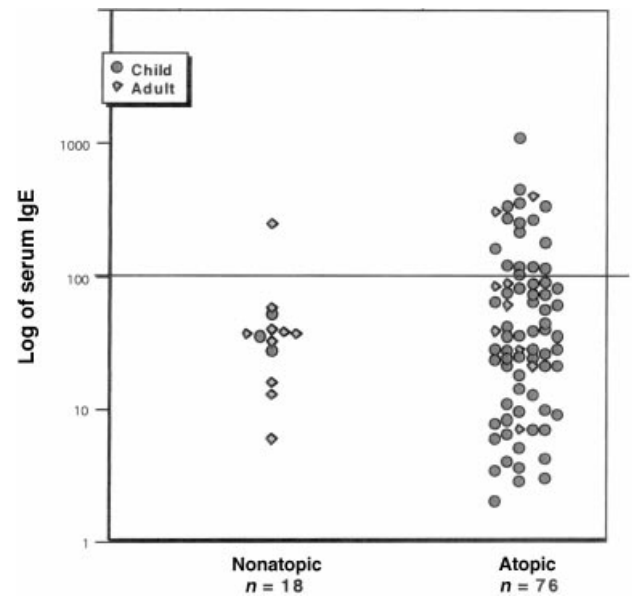


Figure 4. Total serum IgE in 94 patients with OME.

stimuli than neutrophils from nonatopic patients. The total capacity of cells from atopics for oxidative metabolic activity apparently is not abnormal, but perhaps reflects a greater responsiveness to low levels of stimulation. Styrt et al. (17) found that "the neutrophils from atopics may be both easier to stimulate and more difficult to suppress than cells from normals". One explanation for the increased MPO we report in MEE is that an increase in neutrophils may occur as a result of weak stimulation of these cells as bacteria are being cleared from the site of inflammation (18). Recent studies have indicated that bacterial mRNA present in otherwise "sterile" MEE may serve as a stimulus to T-cell activation (19). Atopy may thus contribute to the elevated levels of MPO seen in Fig. 2. Nevertheless, the elevations we report concur with histopathologic studies which have demonstrated that eosinophils and neutrophils are integral components in OME infiltrates (20, 21).

Specific and total IgE

Quantitative ELISA is now an important tool for providing objective measurements of environmental allergen exposure and for determining allergen standardization (22). There is good correlation between the results of the ThabestTM assay, diagnostic skin tests, and RAST (8, 7, 23). Thabest proved to have greater sensitivity than RAST in 23 patients for whom both assay methods were used, especially in those children with total IgE of <30.

Prior studies which led otolaryngologists to believe that fewer than 30% of OME cases were related to allergy were based on definitions of atopy requiring both rhinitis and IgE of >100 µg/l (24), or results of prick testing. Our data show that the mean IgE among atopics was 93.8 µg/l, two-thirds of atopics with OME

having serum IgE of <100 $\mu\text{g/l}$ (Fig. 4). Otitis is thus similar to rhinitis in having no relation to total IgE, and is unlike asthma, which does show correlation (25). Our reported prevalence of atopy among all OME patients of 81% reflects the increased sensitivity of *in vitro* testing. This percentage is consistent with prior reports based on *in vitro* testing by McMahan et al. (26) (88%), Tomonaga et al. (27) (72%), Nsouli et al. (28) (86%), and Corey et al. (29) (60%).

The allergic subject is known to be more prone to develop OME following acute otitis than a nonatopic child (30, 31). In addition to this increased susceptibility, the fact that 93% of the 97 children with persistent effusion were atopic suggests that the different cellular response we documented in atopics results in more chronic disease and constitutes an important justification for the need to develop a new treatment approach for this disease. Just as an atopic child may be more likely to develop asthmatic bronchitis after a viral infection, so, too, an atopic with otitis may be more prone to develop chronic effusion. Studies have suggested that reducing the incidence of OME may depend on controlling the patient's allergies (26, 28, 32, 33). Certainly, many

children in day care get upper respiratory infections and acute otitis media. Perhaps it is predominantly those who are atopic who progress to chronic OME. The observation that the inflammatory pattern in the middle ears of atopics differs markedly from that seen in nonatopics (Figs. 1–3) deserves further investigation. The paucity of conventional randomized clinical trials dealing with OME, especially those investigating how it might relate to allergy, clearly indicates the need to devise alternate methods to study this disease. There is a need to develop outcome research instruments and methods to validate the efficacy of various treatments, including allergy management.

In conclusion, the inflammatory response by eosinophils, neutrophils, and mast cells in the middle ear was distinctly different between atopic and nonatopic patients ($P < 0.001$). These findings provide further evidence that eosinophils and mast cells, both essential to a Th-2-driven immune response, are active in the majority of ears from atopics with chronic OME. They support the hypothesis that middle-ear mucosa, like that of the rest of the upper respiratory tract, is capable of an allergic response. OME in children may be an allergic disease.

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