ORIGINAL ARTICLE

The Impact of Atopy on Neutrophil Activity in Middle Ear Effusion From Children and Adults With Chronic Otitis Media

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Objective: To identify the relationship of neutrophil activity to allergy as reflected by the level of myeloperoxidase (MPO) in ears of atopic patients with chronic otitis media with effusion (OME) by objective testing.

Design: Evidence of neutrophils was measured in the effusion of atopic patients with chronic OME. Atopy was determined by intradermal and/or in vitro testing of allergic reaction to 10 inhalants, 2 molds, and 5 foods.

Subjects: Effusion MPO was measured prospectively in 138 ears from 106 consecutive patients with chronic OME.

Results: A total of 86 (81%) of 106 patients with OME tested atopic by in vitro or in vivo testing. Excluding 36 ears with purulence, the mean MPO level was 3132 µg/L in 84 atopic vs 142 µg/L in 18 nonatopic ears ($P<.001$). A total of 78 (90%) of 87 patients with OME were atopic.

Conclusions: The surprising finding of marked elevation of effusion MPO in atopic patients but very low levels in nonatopic patients ($P<.001$) suggests that atopy may contribute to elevated levels of neutrophil activity in OME. An atopic patient may respond differently from a nonatopic one to the microbial or viral products of acute inflammation owing to the presence of primed inflammatory cells. This study provides confirmation on a cellular level that neutrophils are an integral part of the inflammatory process in OME to a disproportionate degree among atopic patients.

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IDENTIFICATION OF the factors responsible for the chronic nature of otitis media is an essential step in developing treatment and ultimate prevention strategies for this disease. Histopathologic studies have demonstrated that neutrophils and eosinophils are integral components in middle ear infiltrates.[1]

A role for the neutrophil in the allergen-induced inflammatory process seems counterintuitive because the presence of neutrophils, unlike that of eosinophils, is not normally associated with an atopic helper T–cell (T_H) 2 inflammatory response. The predominance of neutrophils in middle ear effusion(MEE) thus serves as a major refutation of the hypothesis that allergy might be a significant contributing factor to the pathogenesis of otitis media with effusion (OME) and supports the theory that chronic otitis is predominantly a response to infection. However, it is disturbing to recognize that after 25 years of working under the "infection hypothesis," evidence-based medicine confirms that otitis media resolves within 2 months of initial infection in 70% of children regardless of antibiotic treatment.[2] Among those 30% of children in whom it progresses to chronic OME, more than 85% have been proven atopic by objective testing.[3,4] The objectives of this study were (1) to investigate the relationship of neutrophil activity to allergy, as reflected by the levels of myeloperoxidase (MPO) in ears with OME, and (2) to determine if there is a difference in the inflammatory response in an OME ear in atopic vs nonatopic patients, as determined by objective in vitro or in vivo testing.

RESULTS

The demographic characteristics regarding age distribution and atopic status are given in Table 1. Eighty-five of the 106 patients were classified as having only refractory, nonacute OME; 15 patients had signs of a recent infection (PUR); and 6 more presented with 1 PUR and 1 non-PUR ear.

ATOPIC STATUS

A total of 86 (81%) of 106 patients with OME were atopic by in vitro or in vivo testing. Among the 19 adults the prevalence

SUBJECTS AND METHODS

EFFUSION SUBJECTS

To characterize the relationship of the neutrophil response to allergy or infection in OME, we measured MPO levels in effusion from 106 individuals who presented with refractory effusion to a solo-practitioner, community-based otolaryngologist. Fifty-one young children (aged 14 months to 6 years), 36 children of school age (6-18 years), and 19 adults were selected in a consecutive, prospective manner. None was immunodeficient or exhibited congenital malformations. All had documented hearing loss, flat tympanograms, and effusion of a minimum of 3 months' duration that was unresponsive to antibiotic and/or decongestant therapy. Middle ear effusions were collected at the time the patients underwent routine myringotomy and placement of tympanostomy tubes (M&T). The patients were tested for allergy only after they were entered into the study to avoid preselection bias. The study proceeded following approval of the Franklin Memorial Hospital (Farmington, Me) Committee on Ethics and Human Experimentation and patient or parental consent.

The effusion from 138 ears, including 32 pairs, was collected quantitatively in a Juhn Tym-Tap (Xomed, Jacksonville, Fla) and diluted with precisely 2 mL of isotonic sodium chloride solution. Supernatants of diluted, centrifuged specimens were pipetted, stored at –20°C, and later tested for MPO. Variation in the volume of middle ear fluid was previously considered by measuring 14 samples containing lithium chloride. Effusion volumes collected by our method were quite similar, ranging from 0.11 to 0.43 mL (mean ± 2 SDs, 0.32 ± 0.11 mL).[5] 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 prior to their M&T. These patients represent a mixed, purulent type of otitis (PUR). Any patient with pus in his or her effusion, or even a hyperemic tympanic membrane at the time of myringotomy, was included in this PUR group and evaluated separately. Ears that typified episodes of recurrent acute otitis media that quickly resolved 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.[5] The results were sorted by patient type (ie, atopic vs nonatopic) as well as by MPO, total serum IgE, and age (**Table 1** and **Table 2**).

DIAGNOSIS OF ATOPY

After undergoing M&T, all patients in both groups were evaluated for allergies by in vitro and/or in vivo testing for specific IgE with a battery of 15 allergens (dust mites der P and der F, cat, dog, Timothy grass, short ragweed, birch, oak, *Alternaria*, *Hormodendrum*, milk, wheat, corn, soy, and egg). Patients were categorized as atopic if they reacted positively to at least 2 antigens at a modified radioallergosorbent test (RAST) class 2 or higher by either testing method. This definition resulted in an intentional bias to exclude borderline atopic patients.

In vitro testing used either RAST (Immuno-CAP; Pharmacia-Upjohn, Uppsala, Sweden) or Thabest (Integrative Medicine Inc, Denville, NJ) IgE micro–enzyme-linked immunosorbent assay (micro-ELISA) testing of serum. Intradermal skin testing for the same battery of 10 inhalants, 2 molds, and 5 foods was performed by injection of an allergenic extract in volumes of 0.01 mL to produce a 4-mm wheal. The skin tests were performed with 1:500 wt/vol or serial 5-fold weaker dilutions of the allergenic extracts. Results were considered positive when a wheal diameter of 7 mm or larger was observed that was at least 2 mm larger than the wheal from a glycerin control of the same dilution strength after 10 minutes.

IN VITRO TESTING

Serum RAST or micro-ELISA testing was performed for specific serum IgE in all patients. Atopy was determined without knowledge of the mediator results so as to have a single-blind study.

TITRATION OF MEDIATORS IN EFFUSION

Myeloperoxidase levels were measured by a double antibody radioimmunoassay (Pharmacia-Upjohn Diagnostic AB, Uppsala, Sweden) according to the instructions of the manufacturer. The MPO interassay coefficient of variation varied between 6% and 10%, and levels under 8 µg/L were undetectable. The stability of MPO in middle ear fluid was verified by incubating a known amount of MPO (8-1000 µg/L) into 1 of the ear samples for 1 hour. This was then assayed for the protein. The mean ± 2 SDs recovery rate for MPO was 102.9% ± 5.3% (n = 7). Effusion mediator levels were considered abnormally elevated if the MPO level was greater than 407 µg/L (ie, the nonatopic mean + 2 SDs). Initially, MPO levels were measured using radioimmunoassay technique at a research facility in Sweden.

STATISTICAL ANALYSIS

Statistical analyses were carried out by means of nonparametric tests. The Mann-Whitney *U* test with the Bonferroni correction was used to compare the different groups (atopic vs nonatopic). Results are given as mean ± SEM. Statistical calculations were performed using the InStat statistical package (GraphPad Software Inc, San Diego, Calif) with a Power Mac 7200 personal computer (Apple Computer Inc, Cupertino, Calif).

of atopy at 42% (8/19) was higher than that found in the general population. Among the children, 78 (90%) of 87 were atopic (Table 1). Evaluation of the type and number of antigens to which atopic patients had a positive intratermal skin testing reaction and/or had in vitro analyses that elicited a class 2 or higher response revealed that for inhalants, 37 (42%) reacted to 2 to 5 antigens, and 34 (39%) had antibodies to 6 or more. Additionally, 61 (70%) reacted at class 2 or higher to both molds, 5 (6%) to just 1, and 44 (51%) had significant reactions to foods. Thirty-two children (37%) demonstrated antibodies to 1 or 2 foods and 12 (14%) to 3 to 5 foods. Retrospective

Table 1. Demographics and Myeloperoxidase (MPO) Levels in Nonatopic and Atopic Patients With Various Mediator Levels*

Characteristic	Children			Adults (Age >18 y)	Total Patients	Mean Age, y	MPO Level, µg/L†		
	Age <6 y	Age 6-18 y	Total				≤400	>400	Total
Nonatopic	8	1	9 (9)	11 (58)	20 (19)	38.7	18	0	18
Atopic	43	35	78 (90)	8 (42)	86 (81)	9.1	15	66	81
Total No. of Patients	**51**	**36**	**87**	**19**	**106**	. . .	**33**	**66**	**99**

*Unless otherwise indicated, all data are number or number (percentage) of patients. Percentages may have been rounded. Ellipses indicate not applicable.
†Excluding patients who experienced a superimposed acute ear infection within 2 weeks prior to the routine myringotomy and placement of tympanostomy tubes.

Table 2. Mediator Levels Found in Study Subjects*

	Nonatopic	Atopic	PUR	Total
		Effusion MPO†		
No. of ears	20	84	34	**138**
Mean (SD) level, µg/L	141.65 (132.71)	3132.00 (34.54)	151.40.00 (116.00)	. . .
SEM	32.19	379.00	2026.00	. . .
		Total Serum IgE‡		
No. of patients	20	84	. . .	**104**
Mean (SD) level, µg/L	44.7 (52.94)	91.6 (154.7)	. . .	. . .
SEM	11.8	16.8	. . .	. . .

*PUR indicates a patient who experienced a superimposed acute ear infection within 2 weeks prior to the routine myringotomy and placement of tympanostomy tubes; ellipses, not applicable.
†$P<.001$ for the difference between nonatopic and atopic subjects.
‡$P=.74$ (nonsignificant) for the difference between nonatopic and atopic subjects.

review of the 54 charts, including written inquiry to the referring physician, revealed that 33 (61%) of the 54 children had documentation of additional atopic signs and symptoms including asthma (n=12; 22%), allergic rhinitis (n=26; 48%), eczema (n=2; 4%), and chronic nasal congestion (n=4; 7%). Otitis media with effusion was the sole symptom of allergy in 18 children (33%).

MEDIATOR LEVELS IN EFFUSIONS

The inflammatory response by neutrophils in the middle ear of atopic patients was distinctly different than it was in nonatopic patients (**Figure 1**). The mean MPO level in atopic patients was 3132 µg/L vs 142 µg/L in nonatopic patients ($P<.001$). The highest levels of MPO (mean, 15 140 µg/L) were found in PUR ears at the time of myringotomy. Among non-PUR ears, the mean MPO level of atopic patients was 22 times higher than that of nonatopic subjects (Figure 1, Table 2).

Most atopic patients had a serum IgE level lower than 100 µg/L. Total IgE levels did not differ between atopic (mean, 91.6 µg/L) and nonatopic subjects (mean, 44.7 µg/L; $P=.74$) (Table 2).

COMMENT

Our data indicate a unique response by neutrophils in the middle ear of atopic vs nonatopic subjects. In humans, the influx of neutrophils correlates with levels of interleukin (IL) 8. Many investigators have described IL-8 in middle ear fluid,[1] but the implications of this occurence, especially as might relate to our findings, has not been appreciated. Interleukin 8 controls initiation and maintenance of the inflammatory process in various tissues and acts as a chemotactic cytokine for neutrophils and primed eosinophils.[6] Significant sources of IL-8 include endothelial cells, fibroblasts, macrophages, epithelial cells, and primed eosinophils.[7,8] Neutrophils and eosinophils express IL-8 messenger RNA[7] and serve as a source of other cytokines into the site of inflammation. Interleukin 8 affects neutrophils by inducing activation of the motile apparatus, directional migration, expression of surface adhesion molecules, and production of reactive oxygen metabolites and by causing the release of storage enzymes,[9] including MPO. In allergic bronchial asthma and dermatitis, IL-8 is a chemoattractant for T lymphocytes and basophils and induces histamine release from primed basophils.[8] An increase in IL-8 secretion is associated with the nasal allergic reaction after allergen challenge in atopic patients but not controls.[6] Thus, IL-8 activity is amplified in the allergic response (**Figure 2**) and may explain the elevation of neutrophils we recorded in the ears of atopic patients.

Neutrophil involvement is not unknown in other allergic diseases. A difference in neutrophil response has been reported among atopic vs nonatopic humans in allergic asthma, rhinitis, and atopic dermatitis. Neutrophil inflammation contributes to the pathophysiology of asthma under natural allergen exposure.[10] The late asthmatic response in animal models is dependent on neutrophil availability and neutrophil chemotactic factor. Styrt et al[11] found that "the neutrophil from atopics may be both easier to stimulate and more difficult to suppress than cells from normals." In mice, neutrophils are re-

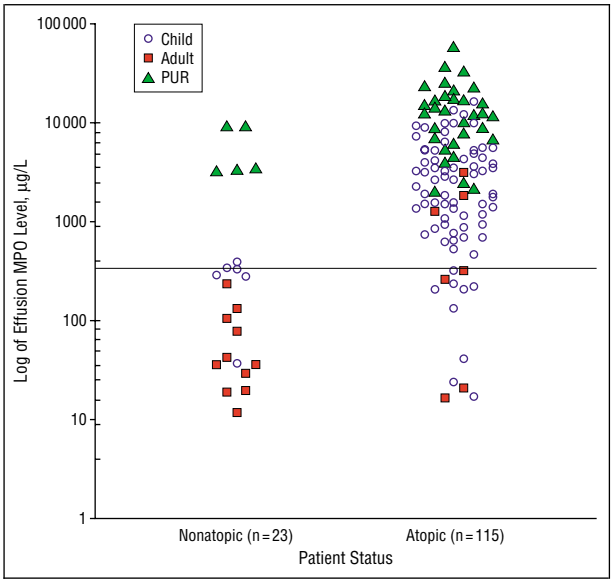

Figure 1. A scatterplot comparison of myeloperoxidase (MPO) levels found in ears of atopic and nonatopic subjects. The horizontal line represents the mean+2 SDs MPO level of nonatopic subjects (400 µg/L). PUR indicates a patient who experienced a superimposed acute ear infection within 2 weeks prior to the routine myringotomy and placement of tympanostomy tubes. $P<.001$ for the difference in MPO levels between atopic and nonatopic subjects.

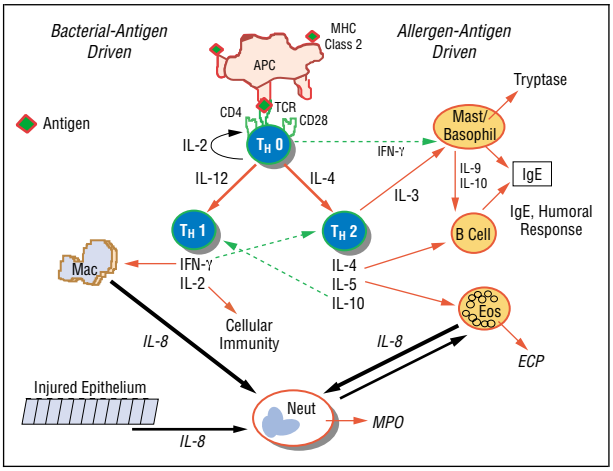

Figure 2. A graphic representation of current concepts of T-, B-, and antigen-presenting cell (APC) interrelationships, with emphasis on neutrophil (Neut) chemotaxis under the influence of interleukin (IL) 8 expressed by macrophages, injured epithelium, and sensitized eosinophils. Bacterial antigen-induced (T_H1) and allergen-induced (T_H2) reactions lead to the release of cell mediators tryptase, eosinophil cationic protein (ECP), and myeloperoxidase (MPO). APCs include macrophages (Mac), B cells, and dendritic cells. IFN-γ indicates interferon gamma; MHC, major histocompatibility complex; T_H, helper T cell; and TCR, T-cell antigen receptor.

cruited to the lung early after allergen challenge, whereas eosinophil recruitment occurs at a later time.[12]

Interleukin 8 also contributes to selective eosinophil recruitment in allergic inflammatory responses in vivo.[8] The eosinophil, an important cell in the late-phase allergic reaction, is both an effector and a responder and has been found in high concentrations in the MEE of atopic patients.[5] Only sensitized eosinophils from atopic patients with asthma seem to respond to IL-8, which has been shown to induce eosinophil migration in a dose-dependent manner in pollen-allergic blood donors but not in nonatopic subjects.[7,13]

In the middle ear, IL-8 may be responsible for prolongation of the inflammatory process, as its concentration in chronic mucoid OME is reportedly 5 times that found in acute purulent otitis.[14] The closed middle ear space seems to act like a trap for IL-8, unlike the lung where the cytokine is not concentrated. Maxwell et al[1] hypothesized that "IL-8 is crucial in the leukocyte response in the middle ear and is pivotal in the maintenance of inflammation in chronic OME." The total number and percentage of neutrophils in MEE correlate with the concentration of IL-8 in MEE.[15] Interleukin 8 is present in MEE from children and adults[9] with no difference reported among mucoid, seromucinous, or serous effusions.[1] The presence of IL-8 is strongly correlated with levels of IL-1b and tumor necrosis factor α, both known inducers of IL-8 production.

One hypothesis offered to explain the observed levels of MPO in MEE of allergic patients at 22 times that of the levels in nonatopic subjects (Figure 1) is that OME is a disease merely associated with atopy. The inflammatory cells in the middle ear of atopic subjects in general are more responsive and produce more cytokines, etc, as is seen in allergic asthma, rhinitis, and dermatitis.[6,13,8] Why this should be remains to be determined.

Regardless of a patient's atopic status, antigens retained in MEE after an acute infection are responsible for activating T cells and subsequent cytokine production,[16] particularly IL-1b, IL-2, IL-6, tumor necrosis factor α, interferon γ, and IL-8.[9,1] Interleukin 8 induces a rapid influx of polymorpholeukocytes into the middle ear, but the inflammation is not sustained, suggesting that the effect is temporary unless continued IL-8 expression is present. Persistent reactivity in atopic subjects with resulting high levels of MPO might result from the high concentration of eosinophils in atopic ears,[5] as IL-8 is constitutively expressed by human resting eosinophils.[7] The eosinophil is not only an effector cell but also takes part actively in a cytokine network and in regulating the immune response. Most important, it seems that only eosinophils from symptomatic, pollen-allergic patients with asthma, not those from nonallergic subjects, respond to IL-8.[13] Eosinophils from patients with symptomatic seasonal rhinitis migrate toward IL-8 in a dose-dependent fashion also.[13] Interleukin 5, a specific activator for eosinophils with no effect on neutrophils or monocytes, seems to act as a cofactor in initiating or enhancing the eosinophil response to other chemotactic factors, including IL-8.[13] Interleukin 5 has recently been demonstrated to be present in the mucosa of patients with chronic OME.[17]

An alternative hypothesis is that OME is due to a true allergic response in which the middle ear, like the nose and lung, participates in a type 1, T_H2 immune–mediated allergic reaction. If the middle ear were to participate in a true allergic response, one would then expect to find the results we report: namely, that mediators from eosinophils and mast cells, capable of epithelial damage, might lead to increased IL-8 release and the attraction of neutrophils uniquely among atopic patients. The

middle ear mucosus is similar to that of the rest of the upper respiratory tract and is capable itself of an allergic response.[18] Effusion and mucosal biopsy studies demonstrate that many of the mediators and cells essential to the production of a T_H2 immune–mediated response, including eosinophil cationic protein, tryptase and/or IL-5 messenger RNA cells, $CD3^+$ T cells,[17] eosinophils,[5] and mast cells[18,19] are all present in ears with chronic OME. Indeed, Labadie et al[20] found in animal studies that allergen-sensitized and -challenged mice respond by producing more middle ear fluids to a secondary stimulus than do nonsensitized and unchallenged animals. They showed that allergen challenge per se did not induce any fluid production, which is in agreement with others.[21] The unique reactivity of atopic ears has also been documented in humans with the demonstration of higher fluid levels of vascular cell adhesion molecule 1 than is found in nonatopic subjects.[22] Messenger RNA for IL-8 is actively produced in the cells found in MEE associated with viral infections but is not found to arise from mucosal cells.[23] Biopsy studies demonstrate a greater number of eosinophils and neutrophils in the mucus than in the mucosa of ears of atopic patients with chronic OME and a significant and similar ratio of neutrophils and eosinophils both in the mucus and mucosa,[24] indicating a proportional influx by both cells into the middle ear of atopic patients.

The tendency of purulence in middle-ear disease to elevate neutrophil mediators[5] was expected and confirmed in children and adults (Figure 1, Table 1). Because of the distortion of MPO levels by purulence, data used to demonstrate a relationship of MPO levels to atopy excluded purulent ears (Table 2).

Clinically, the diagnosis of type 1 hypersensitivity is based on the detection of allergen-specific IgE by means of skin testing and/or in vitro testing. In the present study, 86 (81%) of the 106 patients (78 [90%] of the 87 children) with chronic OME were deemed to be atopic by in vitro (n=65), in vivo (n=41), or both (n=22) testing types. With intradermal testing as the standard, Thabest proved to have a greater sensitivity (92% vs 35%) in detecting atopy than RAST in 23 patients for whom all 3 assay methods were used. This was evident especially among those children whose total serum IgE levels were lower than 30 µg/L.

Among nonpurulent ears, 66 (81%) of 81 atopic patients had MPO levels that exceeded the mean + 2 SDs (407 µg/L) of nonatopic subjects (Table 2, Figure 1), indicating a very significant difference between the groups ($P<.001$). Although MPO was present, its levels were not elevated in all atopic patients, suggesting that an elevated effusion MPO level reflects a local activation of neutrophils, not a general systemic atopic response. Twenty-six patients with paired samples had different MPO values in the opposing ears (eg, patient 16: 1480 µg/L in the right, 5604 µg/L in the left), which also suggests a local response.

Although our study is not an epidemologic study, it clearly shows that OME is predominantly a disease of children (>90% of reported cases) and that it presents differently among adults. Only 8 (42%) of the adults in the present study were atopic vs 78 (90%) of the children. The populations differed. Most adults were seen earlier, perhaps because they were able to self-refer. Twelve of the 19 sought care after a single upper respiratory tract infection or with eustachian tube dysfunction following an airplane ride. Neutrophil response was significantly different between adults and children, whether atopic (980 µg/L vs 3296 µg/L) or nonatopic (64 µg/L vs 274 µg/L) ($P>.01$). The response was generally less pronounced among adults, which might reflect either a less chronic nature of the middle-ear disease or a true muting by age of adult tissue inflammatory response (57% of the adults were older than 50 years). Regardless, even after omitting the adult ears, our observations are statistically unchanged.

Studies 20 years ago that led otolaryngologists to believe that fewer than 30% of OME cases were related to allergy had been based on definitions of atopy requiring both rhinitis and total serum IgE levels higher than 100 µg/L[25] or results of skin-prick testing, the sensitivity of which (43%[26]) is less than chance. Our data show that the mean serum IgE level among atopic patients was 91.6 µg/L, with 61 of 84 atopic patients with OME having a serum IgE level lower than 100 µg/L (Table 2). Otitis is thus similar to rhinitis in having no relation to total IgE, unlike asthma, which does show correlation.[27] These results are in keeping with the high percentage of patients with OME reported to be atopic in other studies (Tomonaga et al,[3] 72%; Nsouli et al,[28] 86%; and Hurst,[4] 87.5%) that used positive skin testing or RAST results to define allergy. We believe this to be a reflection of increased sensitivity and objectivity of modern RAST, ELISA, and intradermal skin testing methods, not selection bias.

Our data support 2 observations that may be keys to understanding the development of OME. First, there is some unique quality associated with being atopic, as it is only atopic patients who have elevated levels of MPO (Figure 1) in addition to the expected T_H2 mediators previously described.[5,17,18] This quality is most likely related to a patient's allergen sensitivity and exposure to that allergen. This may help explain why it is that among those children in day care with an acute otitis, it is predominantly the atopic child who has a 3 to 5 times disproportionate tendency to develop chronic OME.[29,30]

Second, elevated MPO levels in effusion suggests that the inflammatory response in atopic ears is not restricted to those cells and mediators involved in the classic T_H2 allergic reaction in which neutrophils are usually nonparticipants. These excessively increased levels of MPO in atopic patients suggest that the general inflammatory response to putative inciting agents such as bacterial and viral products may be amplified in atopy, perhaps via IL-8. The allergic status of the host deserves as much attention as the virulence of the infecting bacteria or virus and holds a key to successful management of OME.[28,31]

In conclusion, regardless of whether the relationship between allergy and OME is direct or indirect, marked elevation of effusion MPO levels in atopic patients (Figure 1) but very low levels in nonatopic subjects ($P<.001$) suggests that atopy may contribute to elevated levels of neutrophil activity in OME. A total of 78 (90%) of the

87 children with OME were atopic. This study provides confirmation on a cellular level that neutrophils are an integral part of the inflammatory process in OME to a disproportionate degree among atopic patients.

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