

Similar allergic inflammation in the middle ear and the upper airway: Evidence linking otitis media with effusion to the united airways concept

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Background: Otitis media with effusion (OME) is a chronic inflammatory disease of the middle ear space characterized by the accumulation of fluid. Previous investigations have suggested that the immunopathologic mechanism underlying the development of middle ear effusion in patients with allergy is largely due to the effects of T_H2 mediators. The composition of the inflammatory substrate in the effusions of allergic otitis media is similar to the late-phase allergic response seen elsewhere in the respiratory tract, such as in asthma and in allergic rhinitis. **Objective:** To determine whether the middle ear compartment may be a component of the united airways in allergic disease by comparing the inflammatory profiles of the middle ear to the upper airway.

Methods: Middle ear effusions, torus tubaris (Eustachian tube mucosa at the nasopharyngeal orifice), and adenoidal tissue biopsies were obtained from 45 patients undergoing simultaneous tympanostomy tube placement for OME and adenoidectomy for adenoid hypertrophy. The cellular and cytokine profiles of each site were investigated by using immunocytochemistry (elastase, CD3, major basic protein) and in situ hybridization (IL-4, IL-5, IFN- γ mRNA). Atopic status was determined for each patient by using skin prick testing.

Results: Eleven of the 45 patients with OME (24%) were atopic. The middle ear effusions of atopic patients had significantly higher levels of eosinophils, T lymphocytes, and IL-4 mRNA⁺ cells ($P < .01$) and significantly lower levels of neutrophils and IFN- γ mRNA⁺ cells ($P < .01$) compared with nonatopic patients. The nasopharyngeal tissue biopsies revealed similar cellular and cytokine profiles.

Conclusion: In atopic patients with OME, the allergic inflammation occurs on both sides of the Eustachian tube, both in the middle ear and in the nasopharynx. The results of this

study support the concept that the middle ear may be part of the united airway in atopic individuals. (*J Allergy Clin Immunol* 2004;114:1110-5.)

Key words: Otitis media with effusion, Eustachian tube, adenoid, IL-4, IL-5, IFN- γ , eosinophil, allergy, united airways, inflammation

In the past decade, extensive research has supported the concept of a united airway in which an intimate interconnection exists between the upper and lower airways in allergic disease. The observed allergic inflammation is not confined to a specific target organ but rather is present in continuum with the common airway.

Numerous cross-sectional studies have documented the frequent coexistence of allergic rhinitis and asthma: between 19% and 38% of patients with allergic rhinitis have coexisting asthma, a prevalence rate much higher than that in the general population.^{1,2} To date, no consistent differences between the inflammatory profiles of upper and lower airways have been identified. The nasal mucosa of subjects with allergic rhinitis and the bronchial mucosa of atopic patients with asthma demonstrate similar cellular infiltrates and cytokine profiles, characterized by increased number of eosinophils, mast cells, and T-helper lymphocytes expressing T_H2-type cytokines.³⁻⁵ Therefore, asthma and allergic rhinitis likely represent different clinical manifestations of a single inflammatory airway syndrome.

Epidemiologically, patients with otitis media with effusion (OME) have an increased prevalence of atopic conditions such as allergic rhinitis, eczema, and asthma.⁶⁻¹¹ Previous investigations have suggested that the immunopathologic mechanism underlying the development of OME in patients with allergy is largely attributed to the effects of T_H2 cytokines and their receptors. Sobol et al¹² recently demonstrated significantly higher numbers of eosinophils and T lymphocytes as well as significantly higher levels of IL-4 and IL-5 mRNA⁺ cells in atopic middle ear effusions (MEEs) compared with nonatopic controls. Thus, the composition of the inflammatory substrate in allergic otitis media is similar to the late-phase allergic response seen in other areas of the respiratory

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Abbreviations used

MEE: Middle ear effusion
OME: Otitis media with effusion

tract, such as in chronic sinusitis, allergic rhinitis, and asthma.¹³⁻¹⁵

Given that the middle ear space is an anatomical extension of the airway by way of the Eustachian tube, and given that the middle ear is capable of mounting an allergic inflammation, we propose that the middle ear may be a component of this united airways concept.

Otitis media with effusion is defined as chronic inflammation of the middle ear mucosa characterized by the retention of fluid within the middle ear space. At a prevalence of 15% to 20%,¹⁶ OME represents both a major pediatric health care issue and a substantial economic burden, estimated in the billions of dollars annually.¹⁷ Despite aggressive therapy, the current management of OME is often unsuccessful, and significant numbers of refractory cases require surgical intervention. The possible integration of the middle ear as part of the united airways concept will have major clinical implications for the diagnosis and management of allergic airway diseases.

Therapeutic outcomes studies provide indirect evidence of a common pathologic mechanism whereby local treatment at one site leads to an improvement at the other site. Particularly, successful treatment of allergic rhinitis results in reduced bronchial hyperresponsiveness in patients with concomitant asthma.^{18,19} Therefore, addressing the allergic inflammation in the airway may lead to an improved middle ear response to medical treatment, and ultimately a possible reduction in the number of surgical interventions required.

Although previous studies have examined the inflammatory response of the ear and of the airway separately, our study is the first to investigate the inflammatory profiles in the ear and airway simultaneously. To study the hypothesis that the middle ear is a component of the united airways concept, we compared the cellular and cytokine profiles of MEE to those of the upper airway (namely the nasopharynx) in both atopic and nonatopic children with OME.

METHODS

Study design

Forty-five children (age 2-18 years) undergoing myringotomy, tympanostomy tube placement, and adenoidectomy were prospectively and consecutively recruited for the study undertaken at the Department of Otolaryngology, Montreal Children's Hospital, McGill University. Participation was by parental informed consent, and the study obtained full scientific and ethical approval from the institutional review board.

All patients had documented conductive hearing loss, flat tympanograms, MEE persisting for >3 months unresponsive to antibiotics, and symptomatic nasal obstruction caused by adenoid hypertrophy. Patients were excluded if medications containing antihistamines were used intraoperatively or within the preceding

1 week, or if immunosuppressive agents, including steroids, were used intraoperatively or within the preceding 6 weeks. Other exclusion criteria consisted of a history of acute otitis media in the preceding 3 weeks, frank pus found in the nasopharynx at the time of surgery, or the presence of congenital malformations (ie, cleft palate), known immunodeficiency disorder, or ciliary dyskinesia.

Diagnosis of atopy

Children underwent skin prick tests for 12 common perennial and seasonal allergens: *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, ragweed, grass mix, trees mix, cockroach, dust mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, and cat and dog epithelium. Histamine (1 mg/mL) and saline reactions were appropriate and served as positive and negative controls. All testing was performed on the patient's forearm not receiving intravenous fluids, and was performed intraoperatively by 1 investigator.

The results were evaluated after 10 minutes. Wheals ≥ 3 mm in diameter than wheals at the site of the negative control were considered positive. Patients were classified as atopic on the basis of their positive skin reactions to at least 1 allergen, and not on the basis of a suggestive clinical history of asthma or allergic rhinitis. Children <2 years old were not included because diagnosis of atopy by skin testing is less reliable.

Sample collection methods

Middle ear fluid was collected in a Juhn Tym-Taps (Xomed Treace Products, Jacksonville, Fla) at the time of myringotomy and tympanostomy tube placement. During adenoidectomy, a cupped forceps was used to take a 3-mm biopsy specimen of both the adenoid pad and the torus tubaris, which is the nasopharyngeal mucosa near the Eustachian tube opening. The torus tubaris specimen was taken on the ipsilateral side of the middle ear fluid sampled.

For ethical reasons, the Eustachian tube mucosa was not biopsied. Instead, we sampled the torus tubaris, which we thought was the most easily accessible and representative tissue. In addition, biopsies of adenoid tissue were taken only in patients with adenoidal hypertrophy, again for ethical reasons.

Fluid preparation

One milliliter sterile PBS was added to the sample. At the laboratory, the sample was resuspended and transferred to a 15-mL falcon tube and centrifuged at 200g (1500 rpm) for 5 minutes. The pellet was cytospun at 300 rpm for 10 minutes onto frosted slides, and differential cell counts were made by Diff-Quick (Baxter Healthcare Corp, McGraw Park, Ill) staining and microscopic examination. Slides were also prepared for immunocytochemistry and in situ hybridization. For immunocytochemistry, the cytopspins were briefly fixed in a solution of acetone:methanol (60:40), air-dried, and stored at -20°C until further use. For in situ hybridization, the cytopspins were air-dried and fixed in 4% paraformaldehyde for 30 minutes, washed twice for 5 minutes with PBS, kept at 37°C overnight, and stored at -80°C until further use.

Immunocytochemistry

Immunocytochemistry (Figs 1-3) was performed by using the alkaline phosphatase antialkaline phosphatase method, as previously described.²⁰ mAbs major basic protein (gift from Dr R. Mogbel, University of Alberta), CD3, and elastase (both from Dako Cytomation Canada, Mississauga, Ontario) were used to detect eosinophils, T lymphocytes, and neutrophils, respectively. Slides were developed by using Fast Red substrate for alkaline phosphatase. Negative control experiments were performed by replacing the primary antibody with an isotype-matched control.

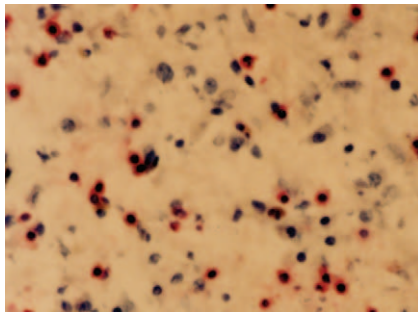


FIG 1. Representative examples of immunocytochemistry for CD3⁺ cells (T cells) in the MEE of an atopic subject. Note the large number of positive cells.

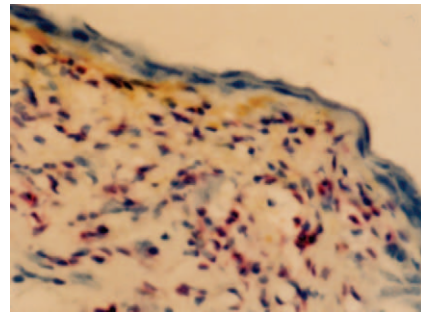


FIG 3. Representative examples of immunocytochemistry for CD3⁺ cells (T cells) in the torus tubaris of an atopic subject. Note the large number of positive cells.

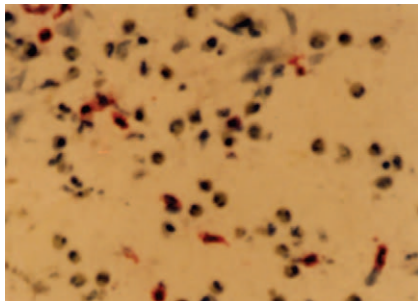


FIG 2. Representative examples of immunocytochemistry for CD3⁺ cells (T cells) in the MEE of a nonatopic subject.

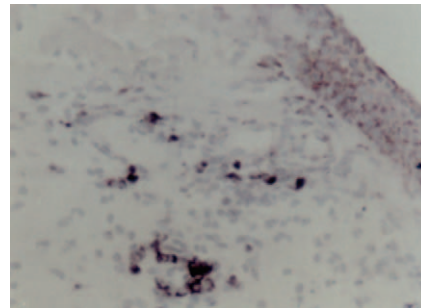


FIG 4. Representative examples of in situ hybridization of IL-4 mRNA in the torus tubaris of an atopic subject. Note the large number of positive cells.

In situ hybridization

Digoxigenin(Dig)-labeled in situ hybridization (Fig 4) was conducted by using digoxigenin-11-uridine-5'-triphosphate-labeled IL-4 and IFN- mRNA riboprobes as previously discussed.²¹ Color development in Dig-labeled in situ hybridization was achieved by adding a freshly prepared substrate solution consisting of 0.175 mg X-phosphate-5-bromo-4-chloro-3-indoly phosphate and 0.37 mg nitroblue tetrazolium salt per milliliter of equalization buffer to the slides (for 20-40 minutes, at room temperature). Slides were transferred to TBS, washed in tap water, and counterstained with hematoxylin for 5 seconds. Positive signals were identified by a purple stain on the cells under light microscopy. Negative control experiments using sense probes and RNase treatment before antisense probe application were performed to confirm probe specificity.

Quantification

For the middle ear fluid, specimens were coded, and the percentage of positive cells for protein or mRNA transcript of interest was counted by using an Olympus microscope (Olympus America, Inc, Melville, NY) with an eyepiece graticule at 200 \times magnification. Results were expressed as the mean percentage of positive cells per cytospin \pm SEM. For the tissue specimens, slides were analyzed for positive signal in a blinded fashion by an investigator by using an Olympus light microscope at 200 \times magnification with an eyepiece graticule of 0.202 mm². The number of positive cells was counted and expressed as the mean per square millimeter. Counting was performed in a blinded fashion by 2 independent examiners.

Statistical analysis

Cell counts were compared between atopic and nonatopic patients by using an ANOVA *t* test, with values of *P* < .01 considered statistically significant.

RESULTS

Atopy of patients with OME

Of the 45 children included in the final analysis, 11 (24.4%) had at least 1 positive skin prick test to any antigen and were classified as atopic (Table I). The remaining 34 (75.6%) children served as nonatopic controls. Table II summarizes the selected demographic and clinical characteristics for patients in each subgroup.

MEE

The percentage of eosinophils and T lymphocytes was significantly higher in the MEE of atopic patients ($7.2 \pm \text{SEM } 0.4$; $12.1 \pm \text{SEM } 1.0$) compared with nonatopic patients ($1.2 \pm \text{SEM } 0.2$; $3.9 \pm \text{SEM } 0.2$; *P* < .01). The percentage of IL-4 and IL-5 mRNA⁺ cells was also significantly higher in atopic patients ($8.4 \pm \text{SEM } 0.7$; $10.5 \pm \text{SEM } 0.9$) compared with nonatopic patients ($1.7 \pm \text{SEM } 0.3$; $1.5 \pm \text{SEM } 0.15$; *P* < .01). See Figs 5-8.

Conversely, a significantly lower percentage of neutrophils and IFN- γ ⁺ cells was found in the MEE of atopic patients ($18.4 \pm \text{SEM } 0.9$; $5.3 \pm \text{SEM } 1.1$) compared with nonatopic patients ($44.4 \pm \text{SEM } 1.3$; $9.3 \pm \text{SEM } 0.7$; *P* < .01). See Figs 9-10.

Adenoid tissue

The number of eosinophils and IL-4 and IL-5 mRNA⁺ cells was significantly higher in the adenoid tissue of atopic patients ($6.4 \pm \text{SEM } 0.9$; $10.3 \pm \text{SEM } 0.9$; $11.1 \pm \text{SEM } 1.0$)

TABLE I. Atopic patients with OME: clinical data

| Patient no. | Sex | Age (y) | Positive skin prick test for allergens |
|-------------|-----|---------|----------------------------------------|
| 1 | M | 4.5 | Dust mite, tree mix |
| 2 | F | 4.9 | Ragweed, cat |
| 3 | M | 5.1 | Dust mite, dog, cockroach |
| 4 | M | 5.4 | Cat |
| 5 | M | 5.5 | Tree mix |
| 6 | F | 5.5 | Dust mite |
| 7 | M | 5.9 | Dust mite, ragweed, tree mix, dog |
| 8 | M | 6.5 | Dust mite, mold |
| 9 | M | 6.5 | Dog, mold |
| 10 | F | 8.8 | Dust mite, cockroach |
| 11 | F | 13 | Dust mite |

TABLE II. Atopic and nonatopic patients with OME: demographic data

| | Atopic group | Nonatopic group |
|----------------|--------------|-----------------|
| N | 11 | 34 |
| Mean age (SEM) | 6.5 y (0.86) | 5.3 y (0.45) |
| Female:male | 1:2.7 | 1:1 |

compared with nonatopic patients ($2.5 \pm \text{SEM } 0.3$; $1.5 \pm \text{SEM } 0.2$; $1.5 \pm \text{SEM } 0.2$; $P < .01$). However, there was no significance difference in T lymphocytes between atopic patients ($51.7 \pm \text{SEM } 2.9$) and nonatopic patients ($59.5 \pm \text{SEM } 1.8$; $P = .06$). See Figs 5-8.

Conversely, a significantly lower number of neutrophils and $\text{IFN-}\gamma^+$ cells was found in the adenoid tissue of atopic patients ($22.9 \pm \text{SEM } 1.2$; $3.3 \pm \text{SEM } 0.6$), compared with nonatopic patients ($34.0 \pm \text{SEM } 1.0$; $6.7 \pm \text{SEM } 0.5$; $P < .01$). See Figs 9-10.

Torus tubaris

The number of eosinophils and T lymphocytes was significantly higher in the torus tubaris tissue of atopic patients ($6.1 \pm \text{SEM } 0.6$; $35.6 \pm \text{SEM } 2.1$) compared with nonatopic patients ($3.1 \pm \text{SEM } 0.2$; $21.1 \pm \text{SEM } 0.7$; $P < .01$). The number of IL-4 and IL-5 mRNA^+ cells was also significantly higher in atopic patients ($8.0 \pm \text{SEM } 1.1$; $8.4 \pm \text{SEM } 1.6$) compared with nonatopic patients ($1.3 \pm \text{SEM } 0.2$; $1.45 \pm \text{SEM } 0.2$; $P < .01$). See Figs 5-8.

Conversely, a significantly lower number of neutrophils was found in the torus tubaris tissue of atopic patients ($26.6 \pm \text{SEM } 1.9$) compared with nonatopic patients ($37.2 \pm \text{SEM } 1.2$; $P < .01$). However, the expression of $\text{IFN-}\gamma^+$ cells in the torus tubaris tissue was not significantly lower in atopic patients ($4.7 \pm \text{SEM } 0.6$) than in nonatopic patients ($6.9 \pm \text{SEM } 0.6$), although there was a trend ($P = .09$). See Figs 9-10.

DISCUSSION

In this study, we found that the MEE of atopic patients had significantly higher levels of eosinophils, T lymphocytes, and IL-4 and IL-5 mRNA^+ cells ($P < .01$) and significantly lower levels of neutrophils and $\text{IFN-}\gamma^+$ mRNA^+ cells ($P < .01$) compared with nonatopic patients.

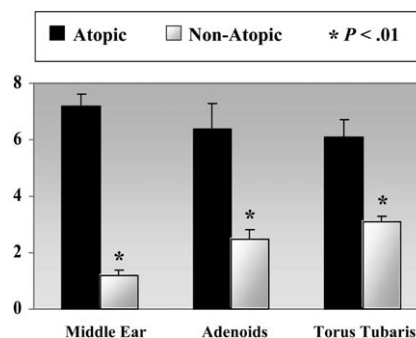


FIG 5. A comparison of eosinophil expression between atopic and nonatopic patients with OME. Middle ear fluid levels measured in percentage of total cells. Torus tubaris and adenoid tissue levels measured in number of cells per square millimeter.

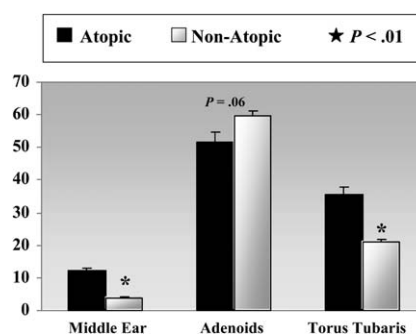


FIG 6. A comparison of T lymphocyte expression between atopic and nonatopic patients with OME. Middle ear fluid levels measured in percentage of total cells. Torus tubaris and adenoid tissue levels measured in number of cells per square millimeter.

The nasopharyngeal biopsies, consisting of torus tubaris and adenoid tissue, also revealed very similar cellular and cytokine profiles. The incidence of atopy among our study population was 24%.

Our data confirm the findings of previous studies suggesting that the middle ear is capable of participating in and sustaining the $\text{T}_\text{H}2$ model of late-phase allergen specific inflammation. Wright et al²² demonstrated increased expression of IL-5 and major basic protein (eosinophils) in the middle ear mucosa of patients with OME compared with normal controls. Hurst and Venge²³ recently reported increased levels of eosinophilic cationic protein in the supernatant of atopic patients with OME compared with nonatopic controls.

For the first time, our study correlates inflammatory profiles found in the MEE to those found in the nasopharynx or the upper airway. Although other studies have examined the middle ear and airway inflammatory profiles separately, no study to date has correlated these findings within a patient. We have shown that the allergic inflammation in atopic children with OME is not isolated to the middle ear. In fact, it occurs uniformly on both sides of the Eustachian tube, in both the middle ear and the nasopharynx. These observed cellular and cytokine profiles resemble the inflammatory substrates in late-phase allergic response previously demonstrated in other areas

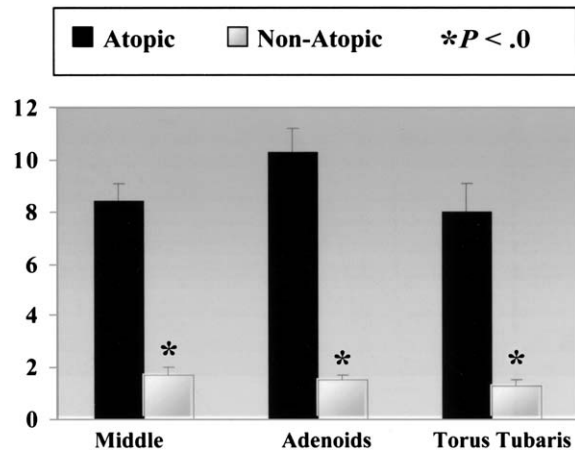


FIG 7. A comparison of IL-4 mRNA expression between atopic and nonatopic patients with OME. Middle ear fluid levels measured in percentage of total cells. Torus tubaris and adenoid tissue levels measured in number of cells per square millimeter.

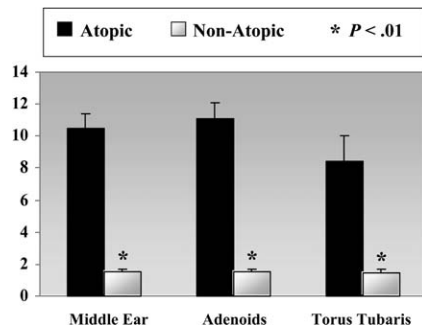


FIG 8. A comparison of IL-5 mRNA expression between atopic and nonatopic patients with OME. Middle ear fluid levels measured in percentage of total cells. Torus tubaris and adenoid tissue levels measured in number of cells per square millimeter.

of the respiratory tract and implicated in the pathogenesis of asthma, allergic rhinitis, and allergy-associated chronic sinusitis.¹³⁻¹⁵ Our data support the concept that the middle ear may be part of the united airway and may behave in a similar fashion to the lungs under allergic inflammatory insults.

In the recent years, there are growing data to suggest that the mechanism for the upper-lower airway interaction occurs through shared systemic inflammatory processes. In a study by Braunstahl et al,²⁴ subjects with allergic rhinitis underwent nasal allergen provocation testing. They showed an induced increase in eosinophil and vascular adhesion molecule levels in both nasal and, interestingly, bronchial biopsies. Conversely, segmental bronchial provocation induced nasal inflammation in patients with allergic rhinitis without asthma.²⁵

In this proposed systemic pathophysiological pathway, the site of disease manifestation demonstrates a severe local inflammatory response, followed by a more generalized inflammatory reaction in the remaining airway. This pathway may possibly explain the interaction between the middle ear and the remaining airway. In our study, the

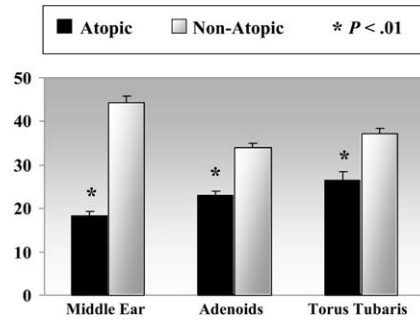


FIG 9. A comparison of neutrophil expression between atopic and nonatopic patients with OME. Middle ear fluid levels measured in percentage of total cells. Torus tubaris and adenoid tissue levels measured in number of cells per square millimeter.

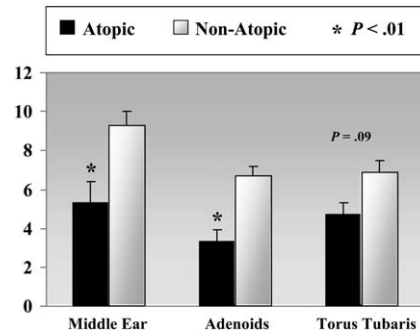


FIG 10. A comparison of IFN- γ mRNA expression between atopic and nonatopic patients with OME. Middle ear fluid levels measured in percentage of total cells. Torus tubaris and adenoid tissue levels measured in number of cells per square millimeter.

increase in eosinophils in atopic patients (compared with nonatopic patients) is much greater in the MEE than that seen in the torus tubaris or adenoid tissue. These results suggest that, in addition to participating in a generalized allergic inflammation of the airway, the middle ear is capable of a more intense local inflammation. This may be caused by superimposed local factors in the middle ear, such as chemokines, which can be produced by structural cells.

In contrast with atopic patients, nonatopic patients revealed different cellular and cytokine profiles in the MEE and nasopharynx, with a predominantly T_H1 -mediated inflammatory response. Although at significantly lower levels, the presence of neutrophils and IFN- γ in atopic patients may represent the residual effects of a previous infectious process, despite the fact that patients with purulent effusions, pus in the nasopharynx, or a history of a recent ear infection were excluded. As well, within the atopic patients, another possible explanation for the low levels of IFN- γ levels seen is that there is suppression of the basal levels of IFN- γ by the increased levels of IL-4, thereby maintaining a balance of T_H1/T_H2 cytokines.

In summary, we have shown for the first time that the allergic inflammation seen in atopics with OME is not isolated to the middle ear but occurs on both sides of the Eustachian tube, in both the middle ear and the nasopharynx.

This inflammation is typically seen in the rest of the respiratory tract in allergic diseases. Therefore, our data support the concept that the ME may be included in the united airways. These findings may have major clinical implications for OME evaluation and management. An integrated management approach to allergic OME should take into consideration the common underlying systemic inflammation and the unity of airways. As the incidence of allergy in children continues to rise, these issues will play an increasingly important role.

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REFERENCES

1. Blair H. Natural history of childhood asthma: 20-year follow-up. *Arch Dis Child* 1977;52:613-9.
2. Pederson PA, Weeke ER. Asthma and allergic rhinitis in the same patients. *Allergy* 1983;38:25-9.
3. Sobol SE, Fukakusa M, Christodouloupoulos P, Manoukian JJ, Schloss MD, Frenkiel S, et al. Inflammation and remodeling of the sinus mucosa in children and adults with chronic sinusitis. *Laryngoscope* 2003;113:410-4.
4. Min YG, Lee CH, Rhee CS, Hong SK, Kwon SH. Increased expression of IL-4, IL-5, IFN-gamma, IL-6, IL-8, and TGF-beta mRNAs in maxillary mucosa of patients with chronic sinusitis. *Am J Rhinol* 1999;13:339-43.
5. Sugita M, Kuribayashi K, Nakagomi T, Miyata S, Matsuyama T, Kitada O. Allergic bronchial asthma: airway inflammation and hyperresponsiveness. *Intern Med* 2003;42:636-43.
6. Hall LJ, Asuncion J, Lukat M. Allergy skin testing under general anesthesia with treatment response in ninety-two patients with chronic serous otitis media. *Am J Otol* 1980;2:150-7.
7. Sorensen CH, Holm-Jensen S. Middle ear effusion and risk factors. *J Otolaryngol* 1982;11:46-51.
8. McMahan JT, Calenoff E, Croft DJ, Barenholtz L, Weber LD. Chronic otitis media with effusion and allergy: modified RAST analysis of 119 cases. *Otolaryngol Head Neck Surg* 1981;89:427-31.
9. Bernstein JM. The role of IgE-mediated hypersensitivity in the development of otitis media with effusion: a review. *Otolaryngol Head Neck Surg* 1993;109:611-20.
10. Mogi G, Tomonaga K, Watanabe T, Chaen T. The role of type I allergy in secretory otitis media and mast cells in the middle ear mucosa. *Acta Otolaryngol Suppl* 1992;493:155-63.
11. Bernstein JM, Tsutsumi H, Ogra PL. The middle ear mucosal immune system in otitis media with effusion. *Am J Otolaryngol* 1985;6:162-8.
12. Sobol SE, Taha R, Schloss MD, Mazer BD, Manoukian JJ, Tewfik TL, et al. T(H)2 cytokine expression in atopic children with otitis media with effusion. *J Allergy Clin Immunol* 2002;110:125-30.
13. Robinson DS, Hamid Q, Ying S, Tscopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
14. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;148:2390-4.
15. Hamilos DL, Leung DY, Wood R, Cunningham L, Bean DK, Yasrael Z, et al. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. *J Allergy Clin Immunol* 1995;96:537-44.
16. Zielhuis GA, Rach GH, van den Broek P. Predisposing factors for otitis media with effusion in young children. *Adv Otorhinolaryngol* 1988;40:65-9.
17. Cypress BK. Patterns of ambulatory care in pediatrics: the National Ambulatory Medical Care Survey. *Vital Health Stat* 13 1983;75:1-60.
18. Corren J, Adinoff AD, Buckmeier AD, Irwin CG. Nasal beclometasone prevents the seasonal increase in bronchial responsiveness in patients with allergic rhinitis and asthma. *J Allergy Clin Immunol* 1992;90:250-6.
19. Watson WT, Becker AB, Simons FER. Treatment of allergic rhinitis with intranasal corticosteroids in patients with mild asthma: effect on lower airway responsiveness. *J Allergy Clin Immunol* 1993;91:97-101.
20. Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. *Am J Respir Cell Mol Biol* 1993;8:35.
21. Ying S, Durham SR, Barkans J, Masuyama K, Jacobson M, Rak S, et al. T cells are the principal source of interleukin-5 mRNA in allergen-induced rhinitis. *Am J Respir Cell Mol Biol* 1993;9:356.
22. Wright ED, Miotto D, Giguere C, Hamid Q. Increased expression of major basic protein (MBP) and interleukin-5(IL-5) in middle ear biopsy specimens from atopic patients with persistent otitis media with effusion. *Otolaryngol Head Neck Surg* 2000;123:533-8.
23. Hurst DS, Venge P. Evidence of eosinophil, neutrophil, and mast-cell mediators in the effusion of OME patients with atopy and without atopy. *Allergy* 2000;55:435-41.
24. Braunstahl GJ, Overbeek S, Kleinjan A, Prins JB, Hoogsteden H, Fokkens W. Nasal allergen provocation induces adhesion molecules expression and tissue eosinophilia in upper and lower airways. *J Allergy Clin Immunol* 2001;107:469-76.
25. Braunstahl GJ, Kleinjan A, Overbeek SE, Prins JB, Hoogsteden HC. Segmental bronchial provocation in allergic rhinitis patients. *Am J Respir Crit Care Med* 2000;161:2051-7.