inducing eosinophilic inflammation in the sinus mucosa or nasal polyps.[9] In asthmatic patients, a specific immunologic response to *S aureus* enterotoxins is associated with clinical and immunologic parameters of asthma severity.[10] In addition, sensitization against fungi, such as *Aspergillus, Alternaria,* and *Cladosporium,* is associated with severe persistent asthma in adults.[11]

The present study was performed to investigate the presence of inhalant and bacterial antigen-specific IgE in MEE of patients with EOM. We also investigated the effect of antigen-specific IgE in MEE on the severity of EOM in each patient.

Methods

Patients

Twenty-six patients (13 women and 13 men; mean [SD] age, 54.3 [12.3] years; age range, 30–78 years) with EOM associated with bronchial asthma were included in this study (EOM group). They were diagnosed as having EOM on the basis of previously reported diagnostic criteria.[5] Patients with EOM were asked about past and present history, including allergic diseases and gastroesophageal reflux symptoms, using a detailed questionnaire. If a patient had a bacterial infection and findings of cytologic or histologic examination of the effusion sample revealed neutrophils rather than eosinophils, the effusion was reexamined to detect eosinophils after controlling the infection. Nine patients with otitis media with effusion and without any history or signs of allergic disease were also included in the study as controls (control group). Control patients were also asked about past and present history using a detailed questionnaire along with physical examination and local findings of nose, throat, and skin to exclude allergic diseases, such as atopic dermatitis, bronchial asthma, nasal allergy, and rhinosinusitis. The cytologic examination revealed no eosinophils on the smear specimen of MEE taken from control patients. Baseline characteristics of the patients in both groups are given in Table 1. All the patients in the EOM group continued their previous treatments for bronchial asthma and EOM during the study. They were all treated with inhaled corticosteroids with or without long-acting β_2-adrenergic agonists for bronchial asthma. For EOM, intratympanic instillation of triamcinolone acetonide was used as the baseline treatment when MEE or otorrhea recurred. When bacterial infection was evident in MEE, oral antibiotics were administered to the patient. Written informed consent was obtained from all patients before sampling of peripheral blood and MEE. The Ethics Committee of Jichi Medical University, Saitama Medical Center, approved this study.

Measurements of Total and Antigen-Specific IgE in Serum

We measured the concentration of total IgE in serum. Identification of the presence of antigen-specific IgE in serum was performed using a multiallergen simultaneous screening test (Phadia, Uppsala, Sweden). This test included 33 antigen-specific IgEs against the following antigens: 10 perennial antigens, such as house dust, mites, *Aspergillus, Alternaria, Candida, Mucor, Penicillium, Cladosporium,* cat skin, and dog skin; 9 pollen antigens, such as cider, birch, and orchard grass; and 14 food allergens, such as egg, wheat, and crab. Antigen-specific IgEs against *S aureus* enterotoxins A and B were not included.

Table 1
Baseline characteristics of the study patients characteristic

	EOM group (n = 26)	Control group (n = 9)
No. of patients	26	9
Female:male sex, No.	13:13	5:4
Age, mean (range), y	54.3 (34–78)	67.6 (29–93)
Total serum IgE level, mean (range), IU/mL	454 (26–1590)	
Positive antigen-specific IgE in serum result, No.		
Perennial antigen(s) only	8	
Perennial and pollen antigens	8	
Pollen antigen(s) only	4	
None	6	

Abbreviation: EOM, eosinophilic otitis media.

Detection of Antigen-Specific IgEs in MEE Samples

The MEE samples were collected with a Juhn Tym-Tap middle ear fluid aspirator/collector (Xomed, Jacksonville, Florida) or forceps if the effusion was too sticky to remove using the former device. One milliliter of saline was added to a total of 50 to 100 mg of an effusion sample, which was weighed to calculate the dilution ratio, and subsequently homogenized into a homogenous fluid at room temperature. Fluid samples were allowed to stand for 60 to 120 minutes and then centrifuged at 1,000 to 1,350g for 10 minutes. The resultant supernatant was collected and stored at −80°C until measurement. Antigen-specific IgEs for detection included mites, *Aspergillus, Alternaria, Candida, Mucor, Penicillium, Cladosporium,* and *S aureus* enterotoxins A and B. Antigen-specific IgEs against pollen, animals, and foods were not tested. The collection of MEE samples was performed at least 1 month after intratympanic administration of corticosteroids.

Bacterial and Fungal Cultures in the Nasopharynx and MEE

Otorrhea or MEE was obtained from each patient for bacterial and fungal cultures. A nasopharyngeal smear was also subjected to bacterial and fungal cultures. Detection of bacteria and fungi was performed on a routine laboratory basis in the clinical microorganism division of our university hospital.

Determination of the Severity of EOM

The severity of EOM was clinically evaluated in 5 items with scores of 0 to 2 as previously reported.[9] The following 3 items were evaluated separately in both ears: the quantity of MEE or otorrhea, the condition of the middle ear mucosa, and the frequency of intratympanic instillation of triamcinolone acetonide. The frequency of administration of systemic corticosteroids and antibiotics to patients was also evaluated. The quantity of MEE or otorrhea was scored as follows: 0, no MEE; 1, MEE with intratympanic aeration when there was no perforation of the eardrum or otorrhea limited to the mesotympanum when there was perforation; and 2, the mesotympanum totally filled with MEE when there was no perforation or otorrhea came out of the mesotympanum to the external auditory canal when there was perforation. The mucosal condition was classified into 1 of 3 categories: 0, nearly normal or slightly edematous; 1, edematous or slightly thickened; and 2, highly thickened or granulated to an extent beyond the position of a normal eardrum. The frequency of intratympanic administration of corticosteroids was scored separately for both ears: 0, none; 1, once in the previous 3 months; and 2, twice or more in the previous 3 months. The frequency of systemic administration of corticosteroids and antibiotics was scored as follows: 0, none; 1, a total of 7 days or less in the previous 3 months; and 2, more than 7 days in the previous 3 months.

Statistical Analysis

Statistical analysis was performed using the χ^2 test, unpaired t test, and Kendall rank correlation coefficient. $P < .05$ was considered statistically significant.

Table 2
Incidence of antigen-specific IgE in middle ear effusion

Antigen	No. (%) of patients	
	EOM group (n = 26)	Control group (n = 9)
Positive for ≥1 antigens	16 (62)[a]	1 (11)[a]
Mite	13	0
Aspergillus	9	0
Alternaria	9	0
Candida	11	0
Mucor	8	0
Penicillium	7	0
Cladosporium	8	0
Staphylococcus aureus		
Enterotoxin A	7	0
Enterotoxin B	5	1
Negative	10	8

Abbreviation: EOM, eosinophilic otitis media.
[a] $P < .01$.

Results

Detection of Total Serum IgE Concentrations and Antigen-Specific IgE in Peripheral Blood

Total serum IgE concentrations were in the range of 26 to 1,590 IU/mL in patients with EOM (mean, 454 IU/mL). One or more antigen-specific IgEs were detected in 20 of 26 patients. Among them, perennial antigen(s), such as mites and house dust with or without pollen antigen(s), were detected in 16 patients. Four patients tested positive for only pollen antigen(s), such as cider pollen (Table 1). The most frequent antigen-specific IgE was against mites (n = 11/26) followed by house dust (n = 9/26). For fungal antigens, only *Candida*-specific IgE was detected in 2 patients.

Detection of Antigen-Specific IgE in MEE

In the EOM group, 1 or more antigen-specific IgEs were detected in MEE from 16 of 26 patients (62%). In contrast, in the control group, only 1 patient (11%) tested positive for antigen-specific IgE against *S aureus* enterotoxin B. The EOM group had a significantly higher prevalence of positivity than the control group ($P < .01$, Table 2).

Total Serum IgE Concentrations and Antigen-Specific IgE in MEE

Patients were divided into 2 groups according to the detection of 1 or more antigen-specific IgEs in MEE: an antigen-specific IgE–positive group (n = 16) and an antigen-specific IgE–negative group (n = 10). Total IgE concentrations in serum were not different between the 2 groups (Fig 1). Perennial antigens, such as house dust and mites, tended to be more frequently found in serum from the antigen-specific IgE–positive group than from the antigen-specific IgE–negative group ($P = .08$). However, prevalence of pollen antigen-specific IgEs in serum was the same in both groups (Fig 2). Among the perennial antigens, mite-specific IgE was detected in the serum of 11 of 13 patients who tested positive for mite-specific IgE in MEE. However, none of the fungi-specific IgEs were detected in serum even though 11 patients tested positive for 1 or more fungal antigens detected in MEE. Two patients with *Candida*-specific IgE in serum tested negative for all antigen-specific IgEs in MEE.

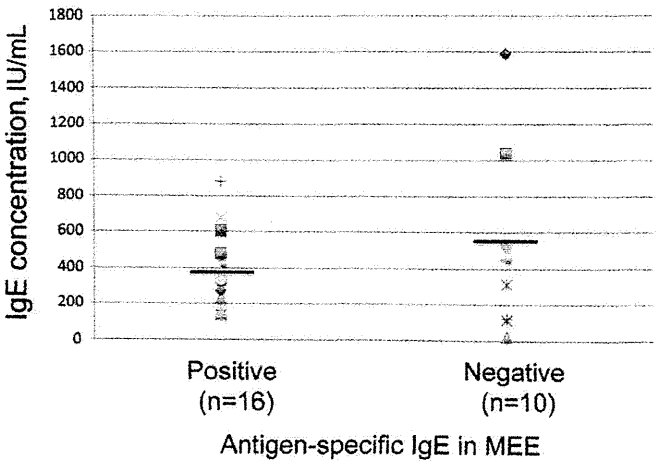

Figure 1. Total serum IgE concentrations in patients with eosinophilic otitis media regarding the presence of 1 or more antigen-specific IgEs in middle ear effusion (MEE).

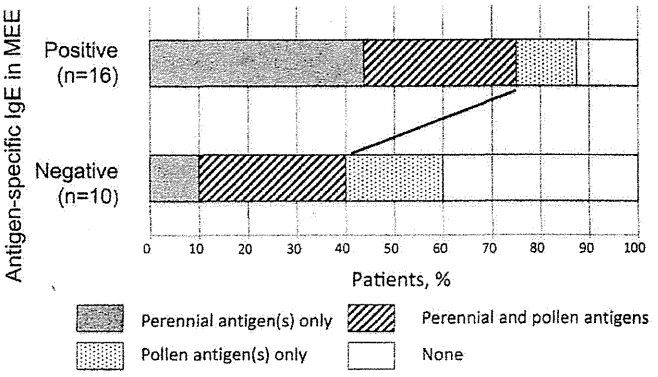

Figure 2. Percentage of patients with perennial or pollen antigen-specific IgE in serum regarding the presence of 1 or more antigen-specific IgEs in middle ear effusion (MEE) ($P = .08$).

Bacteria and Fungi Detected in the Nasopharynx and MEE or Otorrhea

Table 3 gives the results of bacterial and fungal cultures of MEE or otorrhea and nasopharyngeal specimens. Pathogens were detected in MEE or otorrhea samples from 12 patients with EOM. The most frequent bacterium detected was *S aureus*, including 2 patients with methicillin-resistant *S aureus* infection. Only commensal bacteria, such as coagulase-negative *Staphylococcus* and *Corynebacterium*, were detected in MEE or otorrhea samples from 8 patients and nasopharynx samples from 11 patients. *Candida* was detected from otorrhea or nasopharyngeal specimens from 2 patients with EOM. In 7 patients who tested positive for antigen-specific IgE against S aureus enterotoxin A in MEE, *S aureus* was isolated from MEE or otorrhea or nasopharyngeal samples in 4 patients. In 5 patients who tested positive for antigen-specific IgE

Table 3
Bacteria and fungi detected from the MEE and nasopharynx in patients with eosinophilic otitis media

Bacteria or fungi	No. of study patients	
	MEE (n = 25)	Nasopharynx (n = 22)
Pathogen positive	12	7
Staphylococcus aureus	10	1
Methicillin-resistant *S aureus*	2	0
Pseudomonas aeruginosa	3	2
Haemophilus influenzae	0	3
Streptococcus pneumonia	1	0
Candida	1	2
Commensal bacteria only	8	11
Negative	5	4

Abbreviation: MEE, middle ear effusion.

against *S aureus* enterotoxin B in MEE, *S aureus* was isolated from MEE or otorrhea or the nasopharynx in 3 patients. In 11 patients who tested positive for fungi-specific IgEs in MEE, *Candida* was isolated from both MEE or otorrhea and the nasopharynx of 1 patient.

Antigen-Specific IgE(s) in MEE and the Severity Score of EOM

The severity score of EOM was evaluated using 5 items (as described above). The severity score of EOM was significantly higher in the antigen-specific IgE–positive group (mean [SD], 8.8 [3.7]; range, 3–16) than in the antigen-specific IgE–negative group (mean [SD], 4.9 [3.8]; range, 1–13; $P < .05$).

Discussion

We recently reported that high levels of IgE were detected in EOM patients, regardless of the presence of atopic predisposition.[7] Our previous immunohistologic study found that the expression of IgE in middle ear mucosa was found on the surface of mast cells and within plasma cells in EOM patients.[12] These findings suggest that IgE may be locally produced in middle ear mucosa rather than being derived from serum. Not only is EOM is refractory, it also causes deterioration of bone conduction hearing levels, particularly at high frequencies.[13] High tone loss is significantly associated with high concentrations of IgE and eosinophil cationic protein in MEE.[7] Therefore, locally produced IgE may play an important role in the pathogenesis of EOM regarding eosinophilic inflammation in the middle ear and damage of the inner ear.

The present study found that antigen-specific IgEs against some fungal and bacterial antigens were detected in the MEE of EOM patients. Among 13 patients who tested positive for mite-specific IgE in MEE, 11 patients also tested positive for mite-specific IgE in serum and 2 tested negative. This finding indicates that mite-specific IgE may be derived from serum in the former patients. On the other hand, none of the patients who tested positive for fungi-specific IgE in MEE had fungi-specific IgE in their serum. This finding suggests that fungi-specific IgE may not be derived from serum but is locally produced in the middle ear and that the presence of local sensitization against fungi can occur in the middle ear.

Recent collection and culture methods have indicated that fungi are also highly prevalent in nasal mucus from patients with chronic rhinosinusitis with eosinophilic infiltration.[14,15] Fungi have also been histologically found to reside in eosinophilic mucin of sinus samples in most patients with chronic rhinosinusitis using a modification of the conventional Gomori methenamine silver stain.[16] Airborne fungi can induce a T_H2 response and eosinophilic inflammation in the airway in vivo and in vitro.[9,17,18] Okano et al[9] found that dispersed nasal polyp cells produce interleukin 5, interleukin 13, and regulated activation of normal T cells to express and secrete chemokines in response to fungal extracts of *Aspergillus*, *Alternaria*, or *Candida*.

In the lower respiratory tract, sensitivity to fungi, especially *Alternaria* and *Cladosporium* species, is associated with the development, persistence, and severity of allergic asthma. In addition, sensitization to *Aspergillus* is common in severely asthmatic patients.[11] Fairs et al[19] also reported that *Aspergillus* IgE–sensitized patients have elevated neutrophilic airway inflammation and reduced lung function. Therefore, antifungal therapy has recently been used to treat patients with allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitization. This therapy has produced an improvement in asthma control and a reduction in the severity of asthma.[20]

Another important candidate involved in the pathogenesis of EOM may be *S aureus* enterotoxin. We detected *S aureus* enterotoxin–specific IgE in MEE samples from 7 patients, whereas only 1 of 9 control patients tested positive for *S aureus* enterotoxin–specific IgE. There is increasing evidence that microbial superantigens, particularly *S aureus* enterotoxins, are important in amplifying inflammation in atopic and nonatopic patients. Superantigens may also be important in intrinsic asthma because superantigen-producing bacteria may colonize airway epithelial cells. Superantigens produced locally in the airways may lead to class switching of local B cells, resulting in polyclonal IgE production in the airways and also specific IgE against the superantigens.[21]

In chronic rhinosinusitis, *S aureus* is one of the most frequent bacteria colonies in the sinus mucosa and nasal polyps of patients, with an incidence of 60%. Among these patients, 87% had bronchial asthma and aspirin sensitivity compared with 33% in control patients.[22] Furthermore, IgE antibodies to *S aureus* enterotoxin were present in 28% of polyp samples, with a rate as high as 80% in the subgroup with asthma and aspirin sensitivity.[23] In bronchial asthma, a recent meta-analysis of 7 studies found a higher prevalence of *S aureus* enterotoxin sensitization in asthmatic patients than in controls. *S aureus* enterotoxin positivity is significantly correlated with the risk of asthma.[24]

Although we did not measure *S aureus* enterotoxin–specific IgE in serum in the present study, all patients with EOM had disease associated with bronchial asthma, and a high prevalence of *S aureus* enterotoxin–specific IgE in MEE was observed. This finding indicates that *S aureus* enterotoxin sensitization in the middle ear may also be responsible for the pathogenesis of EOM together with sensitization against fungi. Recently, Dutre et al[25] reported that in allergic fungal rhinosinusitis, *S aureus* may synergize with *Aspergillus* species to create a T_H2 tissue signature and add its superantigenic activities to the disease, resulting in the high total IgE concentration. Therefore, *S aureus* coexisting with *Aspergillus* species within the organ, such as sinuses and the middle ear, may play a crucial role in the pathogenesis of eosinophilic upper respiratory tract diseases.

Indeed, we previously reported that risk factors of deteriorating bone conduction hearing levels included high levels of IgE, eosinophil cationic protein in MEE, male sex, the duration of EOM, the severity of middle ear mucosa, and the presence of bacterial infection.[7] Therefore, elimination of bacterial infection, particularly of *S aureus*, may be important in controlling EOM. Inhalant and bacterial antigens may first invade the middle ear via the eustachian tube in asthmatic patients, inducing the production of IgE. Antigens also easily invade the middle ear from the external ear canal with the presence of persistent perforation of the eardrum, resulting in acceleration of inflammation.

In this study, we also found that the presence of antigen-specific IgE in MEE was closely related to the severity of EOM. This finding is consistent with the fact that in eosinophilic inflammatory diseases of the respiratory tract, such as bronchial asthma and nasal polyposis, local production of IgE is a crucial factor for developing and exacerbating eosinophilic inflammation in the middle ear and affecting the severity of EOM. Further studies are necessary to determine whether antifungal or antimicrobial drugs will be able to control EOM.

References

[1] Tomioka S, Yuasa R, Iino Y. Intractable otitis media in cases with bronchial asthma. In: Mogi G, Honjo I, Ishii T, Takasaka T, eds. *Recent Advances in Otitis Media: Proceedings of the Second Extraordinary International Symposium on Recent Advances in Otitis Media*. Amsterdam, the Netherlands: Kugler Publications; 1993:183–186.

[2] Tomioka S, Kobayashi T, Takasaka T. Intractable otitis media in patients with bronchial asthma (eosinophilic otitis media). In: Sanna M, ed. *Cholesteatoma and Mastoid Surgery*. Rome, Italy: CIC Edizioni Internazionali; 1997: 851–853.

[3] Nagamine H, Iino Y, Kojima C, Miyazawa T, Iida T. Clinical characteristics of so-called eosinophilic otitis media. *Auris Nasus Larnyx*. 2002;29:19–28.

[4] Nonaka M, Fukumoto A, Ozu C, et al. IL-5 and eotaxin levels in middle ear effusion and blood from asthmatics with otitis media with effusion. *Acta Otolaryngol*. 2003;123:383–387.

[5] Iino Y, Tomioka-Matsutani S, Matsubara A, Nakagawa T, Nonaka M. Diagnostic criteria of eosinophilic otitis media, a newly recognized middle ear disease. *Auris Nasus Larynx*. 2011;38:456–461.
[6] Iino Y, Kakizaki K, Saruya S, et al. Eustachian tube function in patients with eosinophilic otitis media associated with bronchial asthma evaluated by sonotubometry. *Arch Otolaryngol Head Neck Surg*. 2006;132:1109–1114.
[7] Iino Y, Usubuchi H, Kodama K, et al. Eosinophilic inflammation in the middle ear induces deterioration of bone conduction hearing level in patients with eosinophilic otitis media. *Otol Neurotol*. 2010;31:100–104.
[8] Bachert C, Zhang N, Holtappels G, et al. Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. *J Allergy Clin Immunol*. 2010;126:962–968.
[9] Okano M, Fujiwara T, Haruna T, et al. Role of fungal antigens in eosinophilia-associated cellular responses in nasal polyps: a comparison with enterotoxin. *Clin Exp Allergy*. 2011;41:171–178.
[10] Kowalski ML, Ciesilak M, Perez-Novo CA, Makowska JS, Bachert C. Clinical and immunological determinants of severe/refractory asthma (SRA): association with staphylococcal superantigen-specific IgE antibodies. *Allergy*. 2011;66:32–38.
[11] Knutsen AP, Bush RK, Demain JG, et al. Fungi and allergic lower respiratory tract diseases. *J Allergy Clin Immunol*. 2012;129:280–291.
[12] Iino Y, Nagamine H, Yabe T, Matsutani S. Eosinophils are activated in middle ear mucosa and middle ear effusion of patients with intractable otitis media associated with bronchial asthma. *Clin Exp Allergy*. 2001;31:1135–1143.
[13] Iino Y, Usubuchi H, Kodama K, Takizawa K, Kanazawa T, Ohta Y. Bone conduction hearing level in patients with eosinophilic otitis media associated with bronchial asthma. *Otol Neurotol*. 2008;29:949–952.
[14] Ponicau JU, Sherris DA, Kern EB, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc*. 1999;74:877–884.
[15] Braun H, Buzina W, Greudenshuss K, Beham A, Stammberger H. Eosinophilic fungal rhinosinusitis: a common disorder in Europe? *Laryngoscope*. 2003;113:264–269.
[16] Guo C, Ghadersohi S, Kephart GM, et al. Improving the detection of fungi in eosinophilic mucin: seeing what we could not see before. *Otolaryngol Head Neck Surg*. 2012;147:943–949.
[17] Yamashita Y, Okano M, Yoshino T, et al. Carbohydrate expressed on *Aspergillus fumigatus* induce in vivo allergic Th2-type response. *Clin Exp Allergy*. 2002;113:264–269.
[18] Inoue Y, Matsuzaki Y, Shin S-H, et al. Non-pathogenic, environmental fungi induce activation and degranulation of human eosinophils. *J Immunol*. 2005;175:5439–5447.
[19] Fairs A, Agbetile J, Hargadon B, et al. IgE sensitization to *Aspergillus* fumigatus is associated with reduced lung function in asthma. *Am J Respir Crit Care Med*. 2010;182:1362–1368.
[20] Pasoualotto AC, Powell G, Niven R, Denning DW. The effects of antifungal therapy on severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis. *Respirology*. 2009;14:1121–1127.
[21] Barnes PJ. Intrinsic asthma: not so different from allergic asthma but driven by superantigens? *Clin Exp Allergy*. 2009;39:1145–1151.
[22] Song WJ, Jo EJ, Kang HR, Cho SH, Min KU, Chang YS. Staphylococcal enterotoxin specific IgE and asthma: systematic review and met-analysis. *Asia Pac Allergy*. 2013;3:120–126.
[23] Van Zele T, Gevaert P, Watelet JB, Cuveller C, van Cauwengerge P, Bachert C. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins increased in nasal polyposis. *J Allergy Clin Immunol*. 2004;114:981–983.
[24] Bachert C, van Steen K, Zhang N, et al. Specific IgE against *Staphylococcus aureus* enterotoxins: an independent risk factor for asthma. *J Allergy Clin Immunol*. 2012;130:376–381.
[25] Dutre T, Dousary SAI, Zhang N, Bachert C. Allergic fungal rhinosinusitis: more than a fungal disease? *J Allergy Clin Immunol*. 2013;132:487–489.

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ORIGINAL ARTICLE

Effect of omalizumab on biomarkers in middle ear effusion in patients with eosinophilic otitis media

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Abstract

Conclusions: Eosinophil cationic protein (ECP) concentrations in middle ear effusion (MEE) in patients with eosinophilic otitis media (EOM) were significantly decreased at 3 months after the administration of omalizumab from the baseline level ($p < 0.05$). This study provides new evidence that omalizumab reduces eosinophilic inflammation in the middle ear and that the reduction of ECP may not be caused by suppression of interleukin (IL)-5 production in the middle ear mucosa. *Objective:* EOM is an intractable otitis media characterized by a highly viscous effusion containing eosinophils. We recently reported that anti-IgE therapy using omalizumab was efficacious in the treatment of EOM. To clarify the underlying mechanism, we determined changes in biomarkers in MEE related to eosinophilic inflammation after therapy. *Methods:* Nine patients with EOM received the anti-IgE agent omalizumab for 3 months. Among them, five patients continued anti-IgE therapy for longer than 1 year. Eight EOM patients without administration of omalizumab were also included in the study as controls. The concentrations of eosinophilic inflammatory markers such as ECP, IgE, IL-4, and IL-5 in MEE were measured before and after the administration of omalizumab. *Results:* After 3 months of omalizumab therapy, the ECP concentration in MEE was significantly reduced from the baseline level ($p < 0.05$), while no significant change of ECP in the serum was observed. The concentrations of IL-4 and IL-5 in MEE showed no significant change before and after the therapy in EOM patients treated with omalizumab.

Keywords: *Anti-IgE therapy, eosinophilic cationic protein, ECP, IgE, interleukin (IL)-4, IL-5*

Introduction

Eosinophilic otitis media (EOM) is an intractable otitis media characterized by the presence of a highly viscous yellow effusion containing eosinophils. EOM is mainly associated with bronchial asthma. In 2011, diagnostic criteria for EOM were established by the EOM study group [1]. The major criterion of EOM is otitis media with effusion or chronic otitis media with eosinophil-dominant effusion. The minor criteria are as follows: (1) highly viscous middle ear effusion (MEE); (2) resistance to conventional treatment for otitis media; (3) association with bronchial asthma; and (4) association with nasal polyposis. Definitive cases are defined as positive for the major criterion plus two or more of the minor criteria. However, Churg-Strauss syndrome and hypereosinophilic syndrome are excluded. EOM is an intractable and persistent disease, and it also presents a high risk for development of severe hearing loss. In our previous study, the incidence of deterioration of bone conduction and total deafness was 59% and 6%, respectively [1].

In the middle ear of patients with EOM, active eosinophilic inflammation appears to be present because eosinophil cationic protein (ECP) levels in MEE and the number of EG2 immunopositive cells in the middle ear mucosa are significantly higher in EOM patients than in control patients with common otitis media [2]. Currently, the most reliable

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treatment for EOM is systemic and topical administration of corticosteroids, similar to that for bronchial asthma. Omalizumab, a recombinant humanized monoclonal anti-IgE antibody, is used as an anti-IgE agent with clinical benefits in the treatment of moderate to severe bronchial asthma. Recently, we reported that anti-IgE therapy using omalizumab was efficacious for patients with EOM associated with bronchial asthma [3]. However, the mechanism underlying the effectiveness of omalizumab for EOM has not been determined yet. In this study, we examined changes in some biomarkers related to eosinophilic inflammation in MEE of patients treated with omalizumab to clarify the mechanism.

Material and methods

Patients

Nine patients with EOM who received anti-IgE therapy using omalizumab were included in the present study. The patients were six women and three men aged 33–69 years (mean ± SD, 55.9 ± 11.8 years) at the time of administration of omalizumab (omalizumab group). Eight patients with EOM without anti-IgE therapy were included in the study as controls. All of the patients had bronchial asthma, which had been treated by respiratory specialists. They were diagnosed with EOM by otologists on the basis of previously reported diagnostic criteria [1]. The baseline characteristics of the EOM patients in both groups are shown in Table I. Written informed consent was obtained from all patients before enrolment in the study. This study was approved by the Ethics Committee of Jichi Medical University Saitama Medical Center (study no. RIN 10-11).

Treatments

Omalizumab was given to the nine patients with EOM in the omalizumab group. The dosage of omalizumab was individually determined based on the pretreatment level of total serum IgE and body weight, according to dosing tables that approximately reflected the following formula: 0.016 mg/kg per IU/ml of IgE per 4 weeks. Omalizumab was administered by subcutaneous injections every 2 or 4 weeks. All of the patients in both groups continued their previous treatments for bronchial asthma and EOM during the study (Table I). They were all treated with inhaled corticosteroids with or without long-acting beta 2-adrenergic agonists and oral leukotriene receptor antagonists or suplatast tosilate for bronchial asthma. All of the patients, except for one in each of the groups, had also experienced frequent asthma exacerbations and continuously or frequently taken oral corticosteroids, in addition to inhaled corticosteroid therapy. One patient in the omalizumab

Table I. Baseline characteristics of the patients.

Characteristic	Omalizumab group	Control group	*p* value
No. of patients	9	8	...
Sex (F:M)	6:3	5:3	NS
Age (years, mean ± SD)	55.9 ± 11.8	56.5 ± 14.4	NS
Associated diseases			
Aspirin intolerance	4	3	NS
Chronic rhinosinusitis	9	6	NS
Nasal polyposis	6	6	NS
Bacterial infection in MEE	3	3	NS
Treatments			
Inhaled corticosteroids	9	8	NS
Intranasal corticosteroids	8	7	NS
Intratympanic TA	9	8	NS
Systemic corticosteroids	8	8	NS
Daily	1	1	
Sometimes on exacerbation	7	7	
Leukotriene receptor antagonists	8	7	NS
Suplatast tosilate*	1	1	NS

MEE, middle ear effusion; NS, not significant; TA, triamcinolone acetonide.
*Th2 cytokine inhibitor.

Table II. Total dosage of prednisolone (mg) administered for 1 month before the sampling of middle ear effusion.

Patient	Baseline	3 months	1 year
Omalizumab group			
T.K.	150	150	...
Y.A.*	240	30	0
S.K.*	0	60	0
T.Y.*	30	0	0
Control group			
T.R.	60	60	...
Y.Y.	17.5	75	...

*Patients who continued the omalizumab therapy for more than 1 year.

group had taken 5 mg of prednisolone daily, and one patient in the control group had taken 2 mg of prednisolone daily. The total dosages of prednisolone for 1 month before the sampling of MEE in both groups are summarized in Table II. For EOM, intratympanic instillation of triamcinolone acetonide was used as the baseline treatment when MEE or otorrhea recurred. Systemic administration of antibiotics was given to the patients when bacterial infection was detected in the MEE. Five patients treated by omalizumab continued the therapy for more than 1 year. Although the remaining four patients did not show the complete resolution of EOM, they discontinued the therapy at 3 months for economic reasons.

Preparation of MEE

MEE samples were collected using a Jun-Tym-Tap middle ear fluid aspirator/collector (Xomed, Jacksonville, FL, USA) or using forceps if the effusion was too sticky to remove by the former device. A total of 50–100 mg of an effusion sample was suspended in 1 ml of saline until the fluid became homogeneous using a homogenizer at room temperature. The fluid samples were allowed to stand for 60–120 min and were then centrifuged at 1000–1350 *g* for 10 min. The resultant supernatant was collected and stored at –80°C until measurement of biomarkers.

Measurement of ECP, IgE, interleukin (IL)-4, and IL-5 concentrations in MEE

Concentrations of ECP, IgE, IL-4, and IL-5 in MEE samples were measured by fluorescence enzyme immunoassay. The detection limits of the measurement methods were 2.0 mg/L for ECP, 5.0 IU/ml for IgE, 2.0 pg/ml for IL-4, and 3.9 pg/ml for IL-5.

Measurements of biomarkers in serum

We also monitored the concentrations of ECP and total IgE in the serum before and after therapy. Serum concentrations of ECP and total IgE were measured by fluorescence enzyme immunoassays.

Statistical analysis

Statistical analysis was carried out using the chi-squared test, and paired and unpaired *t* tests. p values < 0.05 were considered significant.

Results

Clinical evaluation of anti-IgE therapy

No adverse events were observed during the administration of omalizumab in any of the patients. Among the nine patients who received anti-IgE therapy, six showed a reduction in MEE. After the 1-year follow-up period, six of nine patients showed a large reduction in the need for intratympanic instillation of triamcinolone acetonide. In contrast, patients in the control group continued their previous therapy and their symptoms did not change.

Concentrations of ECP, IgE, IL-4, and IL-5 in MEE

The concentrations of ECP in MEE at baseline and 3 months after the therapy are shown in Table III. Extremely high ECP concentrations were detected in

Table III. ECP concentrations (μg/L) in middle ear effusion and serum in the omalizumab group and control group.

	Omalizumab		Control	
Factor	MEE	Serum	MEE	Serum
Baseline	64 236 (1853–231 150)	27 (7–86)	18228 (2244–72 154)	19 (10–21)
3 months	33921 (1598–139 000)	21 (3–52)	86012 (2390–348 514)	20 (10–48)
p value	< 0.05	NS	NS	NS

Values shown are average (range) concentrations for nine patients in the omalizumab group and eight patients in the control group. ECP, eosinophil cationic protein; MEE, middle ear effusion; NS, not significant.

Table IV. IgE concentrations (IU/ml) in middle ear effusion and serum in the omalizumab group and control group.

	Omalizumab		Control	
Factor	MEE	Serum	MEE	Serum
Baseline	1588 (65–7438)	252 (57–637)	1004 (36–2010)	302 (37–1110)
3 months	3369 (53–11 319)	603 (168–1300)	964 (256–1359)	300 (48–1000)
p value	NS	< 0.01	NS	NS

Values shown are average (range) concentrations for nine patients in the omalizumab group and eight patients in the control group. MEE, middle ear effusion; NS, not significant.

the MEE of all patients with EOM in both groups. ECP concentrations in MEE were almost 10^3 times those of the serum concentrations in each patient. At 3 months after the administration of omalizumab, ECP concentrations were significantly decreased from baseline levels ($p < 0.05$), while no significant change in serum ECP concentration was noted at 3 months. In the control patients, ECP concentrations at 3 months were the same as those at baseline (Table III).

Table IV shows the change of IgE concentration in MEE and serum in the omalizumab group and the control group. An increase in IgE concentrations in MEE was also found at 3 months, as observed in serum, but this difference was not significant.

No significant change in IL-4 concentrations was observed between baseline and at 3 months of therapy in the omalizumab and control groups (Table V). IL-5 concentrations in MEE were under the detectable limit in two patients at baseline and in four patients at 3 months in the omalizumab group. In the control group, two patients at baseline and one patient at 3 months had IL-5 concentrations less than the detectable limit in the MEE (Table V).

Changes in ECP, IgE, IL-4, and IL-5 in MEE of patients treated by omalizumab for longer than 1 year

Among the five patients who continued the therapy for more than 1 year, one patient showed complete resolution of MEE at 3 months. Although the remaining four patients had reduced recurrence of effusion, samples could be taken when MEE recurred at around 1 year after therapy. The changes in markers of MEE of the four patients are shown in Figure 1. There was no significant change in concentrations of these markers in the four patients during the therapy. A patient with increased levels of ECP and IgE at 1 year was well controlled by omalizumab for bronchial asthma and EOM. However, the effusion recurred at about 1 year. The middle ear mucosa of this case stayed almost normal (grade 1) [3] during the omalizumab therapy. Another case with increased level of ECP was well controlled for asthma but not for EOM, although the incidence of MEE had become less. The middle ear mucosa of the patient was edematous and slightly swollen (grade 2) [3], showing no significant change during the therapy. There was no significant correlation between the systemic administration of prednisolone and the levels of biomarkers. For example, one patient who had received 240 mg of prednisolone at baseline and 30 mg of prednisolone at 3 months showed marked reduction of ECP level at 3 months from the baseline level.

Discussion

The anti-IgE monoclonal antibody using omalizumab has been approved in therapy for moderate to severe bronchial asthma. This antibody binds to free IgE and prevents it from binding to cell surface high-affinity FcεR1 receptors on mast cells, basophils, and

Table V. IL-4 and IL-5 concentrations in middle ear effusion (MEE) in the omalizumab group and control group.

	IL-4 (pg/ml)		IL-5 (pg/ml)	
Factor	Omalizumab	Control	Omalizumab	Control
Baseline	144 (38–466)	175 (39–606)	1261 (UD–6220)	897 (UD–1459)
3 months	239 (27–680)	149 (70–189)	898 (UD–1680)	750 (UD–1816)
p value	NS	NS	NS	NS

Values shown are average (range) concentrations for nine patients in the omalizumab group and eight patients in the control group. IL, interleukin; NS, not significant; UD, under detection limit.

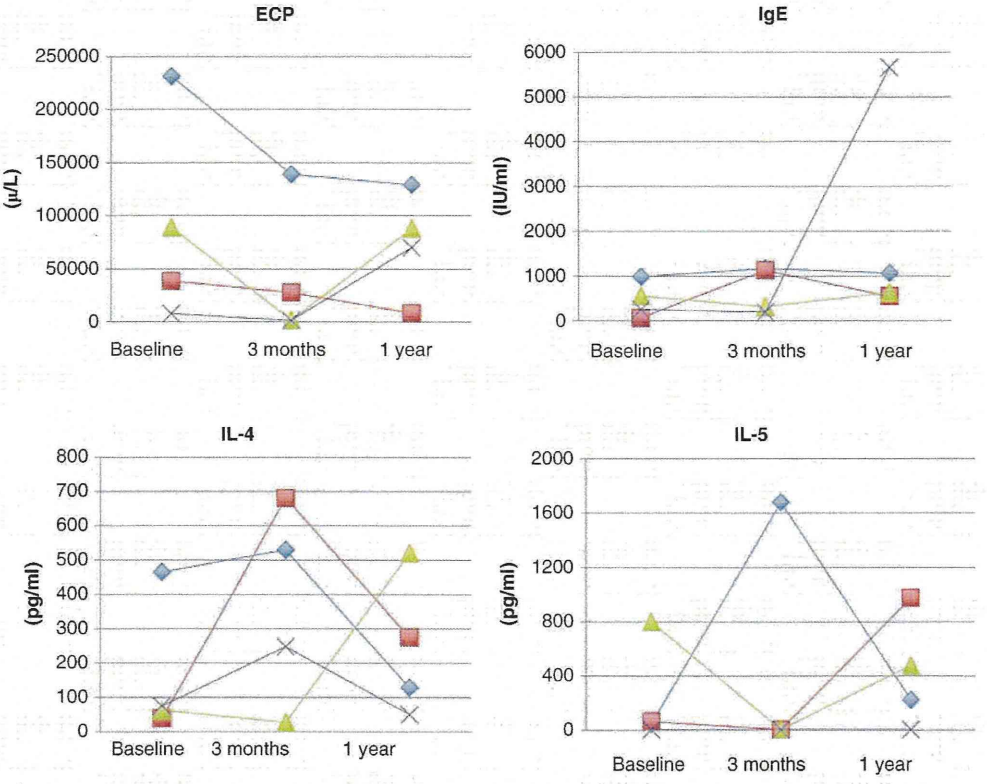

Figure 1. Concentrations of eosinophil cationic protein (ECP), IgE, interleukin (IL)-4, and IL-5 in middle ear effusions of four patients treated with omalizumab for more than 1 year.

eosinophils. This results in IgE cross-linking, and cell activation cannot occur. A large number of studies have shown that omalizumab causes a significant decrease in the number of asthma exacerbations and emergency room visits [4]. A recent meta-analysis of eight trials ($n = 3429$ participants) showed the efficacy and safety of omalizumab in adults, adolescents, and children with moderate to severe asthma [5].

EOM is mostly associated with bronchial asthma, and recent diagnostic criteria for EOM include the association of bronchial asthma as a minor criterion. The effusion of EOM is highly viscous, containing many eosinophils and high concentrations of ECP and IL-5 [2]. We recently showed that significantly higher IgE concentrations were detected in MEE than in serum of EOM patients, regardless of the presence of atopic predisposition [6]. This finding suggests that IgE may be locally produced in the middle ear mucosa rather than being derived from the serum. EOM is not only refractory, but also causes deterioration of bone conduction hearing levels, particularly at high frequencies. High tone loss is significantly associated with high concentrations of IgE and ECP in MEE [6]. Therefore, anti-IgE therapy may also be effective for controlling EOM and for preventing deterioration of hearing.

Recently, we reported the clinical efficacy of anti-IgE therapy using omalizumab for EOM associated with bronchial asthma [3]. Patients who received omalizumab showed improvement in ear symptom scores evaluated by a questionnaire, as well as in clinical scores, including otoscopic findings and systemic and local administration of corticosteroids. In addition, deterioration of bone conduction hearing levels was found less frequently in the omalizumab group than in the control group. In the omalizumab group, one patient showed bilateral deterioration of the bone conduction hearing level beyond 15 dB at the mean speech range or 4 kHz. On the other hand, in the control group, the bone conduction hearing level worsened in seven ears of four patients at the mean speech range or 4 kHz at 1 year. Therefore, anti-IgE therapy is clinically and audiologically effective for EOM, as well as for bronchial asthma.

Eosinophilia is a typical characteristic of asthma-related inflammatory diseases, including EOM. A meta-analysis of five randomized, double-blind, placebo-controlled studies showed that a significant

reduction in the eosinophil count from baseline was observed in patients with allergic asthma treated with omalizumab compared with those administered placebo [7]. In addition to reduction of peripheral blood eosinophils, omalizumab has also been shown to reduce eosinophilia in sputum and bronchial biopsies [8]. In our previous study, the eosinophil count tended to decrease after 3 months of anti-IgE therapy [3]. This reduction in eosinophilia is probably due to a powerful induction of apoptosis of eosinophils. Omalizumab can significantly increase the staining of peripheral eosinophils with apoptotic markers, such as annexin V [9]. The pro-apoptotic action of omalizumab is associated with a decrease in T-cell production of granulocyte macrophage colony stimulating factor (GM-CSF), an important mediator involved in eosinophil growth and survival. GM-CSF is released by $CD4^+$ cells, and plays an important role in late-phase allergic responses that are characterized by eosinophil influx to the airways [9].

The present study showed that ECP concentrations in MEE were significantly decreased after therapy, although the serum ECP concentration failed to show the significant change. This suggested a decreased number of migrating eosinophils in MEE after therapy because ECP is derived from eosinophils and is a marker protein of eosinophilic inflammation. The apoptosis of eosinophils in the middle ear mucosa may occur after the administration of omalizumab. Indeed, the same findings regarding apoptosis of the eosinophils were observed in the middle ear mucosa when triamcinolone acetonide was applied to the middle ear [2].

Total IgE concentrations in the serum was significantly elevated from baseline at 3 months in the present study. MEE concentrations were also slightly elevated after therapy, but this difference was not statistically significant. Total IgE concentrations appear to be increased after the administration of omalizumab because this value includes free IgE, as well as omalizumab-bound IgE [10]. However, a newly available recovery enzyme-linked immunoabsorbent assay for quantifying free serum IgE demonstrated a large reduction in free serum IgE concentrations in patients with severe asthma after therapy [11]. A recent study showed that early elevation of total IgE to more than 250 IU/ml at 3 months from baseline may be used as a predictor of future responders to omalizumab in terms of the exacerbation rate [12].

The most dramatic effect of omalizumab is its ability to reduce levels of the high-affinity IgE receptor FcεR1 in basophils in peripheral blood by approximately 90% in 1 week and by 99% with longer administration [13–15]. Signaling through FcεR1 causes synthesis and release of substantial amounts of Th2 cytokines, such as IL-4, IL-5, and IL-13, which are critical for initiating and maintaining allergic responses. Indeed, omalizumab reduces circulating levels of IL-13, IL-4, and IL-8, in addition to reducing circulating eosinophil counts [16]. IL-4 has been shown to promote class switching to IgE in B cells. In bronchial mucosa, a significantly greater reduction in cells immunostained for IL-4 was observed in the omalizumab-treated group than in the placebo group [8]. IL-13 is also an important cytokine in IgE production and hyper-reactivity in bronchial mucosa of asthmatic patients. Noga et al. [16] reported that omalizumab induced down-regulation of the inflammatory cytokines IL-2 and IL-13, while no significant difference in IL-5, interferon-γ, and tumor necrosis factor (TNF)-α) was observed between the omalizumab-treated group and the control group. In an *in vitro* study, Oliver et al. [17] showed a reduction in IL-4, IL-13, and IL-8 synthesis mediated by FcεR1 in basophils by omalizumab. Roth et al. [18] also showed that omalizumab inhibits the production of IL-4, IL-6, IL-8, and TNF-α in asthmatic airway smooth muscle cells stimulated by IgE. Taken together, these findings suggest that treatment with omalizumab reduces peripheral and bronchial tissue eosinophil counts, as well as diminishing the release of a variety of proinflammatory mediators, such as GM-CSF, IL-2, IL-4, IL-5, and IL-13 [19].

Our previous study also showed that ant-IgE treatment using omalizumab was clinically effective for EOM [3]. To clarify the mechanism, we analyzed some biomarkers in MEE during omalizumab therapy. Our pilot study showed that IL-2, IL-13, and IL-33 concentrations in MEE were under the detectable limit in most of the patients with EOM. Although IL-4 and IL-5 were detected in MEE of patients, no significant changes in their concentrations were observed in MEE during the administration of omalizumab. We previously reported that ECP and IL-5 concentrations are positively correlated in MEE and that locally produced IL-5 may play a crucial role in the accumulation of eosinophils in the middle ear [2]. A significant reduction in ECP concentrations was observed 3 months after omalizumab therapy, regardless of the change in IL-5 concentrations. These results indicate that the reduction of ECP may be due to other mechanisms, such as down-regulation of GM-CSF in mast cells and basophils in the middle ear mucosa.

In conclusion, we provide the first report that ECP concentrations in MEE are significantly reduced after administration of omalizumab. This finding suggests that omalizumab can reduce migration of eosinophils to the middle ear. The effectiveness of omalizumab on EOM may not be caused by suppression of IL-5 production in the middle ear mucosa. Further