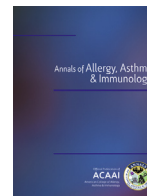




Contents lists available at ScienceDirect



Antigen-specific IgE in middle ear effusion of patients with eosinophilic otitis media

Hiromi Kanazawa, MD; Naohiro Yoshida, MD, PhD; Akihiro Shinnabe, MD; and Yukiko Iino, MD, PhD

Department of Otolaryngology, Jichi Medical University Saitama Medical Center, Saitama, Japan

ARTICLE INFO

Article history:

Received for publication January 6, 2014.

Received in revised form April 16, 2014.

Accepted for publication April 29, 2014.

ABSTRACT

Background: Eosinophilic otitis media (EOM) is an intractable otitis media characterized by a highly viscous effusion that contains eosinophils. We previously reported that high levels of IgE were detected in middle ear effusion (MEE) of patients with EOM.

Objective: To examine antigen-specific IgE in MEE of patients with EOM to clarify the pathogenesis of EOM. **Methods:** Twenty-six patients with EOM associated with bronchial asthma were included in this study. Antigen-specific IgE against inhalant and bacterial antigens were measured in the serum and MEE of these patients.

Results: In patients with EOM, 1 or more antigen-specific IgEs were detected from the MEE of 16 of 26 patients (62%), whereas 1 of 9 control patients (11%) had antigen-specific IgE ($P < .01$). Total serum IgE concentrations were not different between the groups who tested positive (16 patients) and negative (10 patients) for antigen-specific IgE. 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. The severity score of EOM in the antigen-specific IgE-positive group was significantly higher than that in the antigen-specific IgE-negative group ($P < .05$).

Conclusion: Antigen-specific IgE against inhalant and bacterial antigens may be locally produced in the middle ear mucosa in patients with EOM. In particular, local sensitization against fungi together with *Staphylococcus aureus* could result in local IgE production in the middle ear and may be responsible for the severity of EOM.

© 2014 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Eosinophilic otitis media (EOM) is a newly recognized middle ear disease first reported by Tomioka et al¹ in 1994. Since then, there have been several case reports, as well as studies, concerning the pathogenesis of EOM.^{2–4} In 2011, the EOM study group established diagnostic criteria for EOM.⁵ The major criterion of EOM is otitis media with effusion or chronic otitis media with eosinophil-dominant effusion. 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 2 or more of the minor criteria. Strikingly, some patients have gradual or sudden hearing loss, and the incidence of deterioration of bone

conduction and total deafness in patients with EOM is 59% and 6%, respectively.⁵

The cause of eosinophilic inflammation in the middle ear has not been determined. We previously reported that patients with EOM have a significantly longer eustachian tube opening duration compared with that of control patients.⁶ This finding indicates that patients with EOM tend to have insufficient closure of the eustachian tube or a patulous eustachian tube.⁶ Such a condition allows some antigenic material to enter the middle ear via the eustachian tube, causing eosinophilic inflammation with atopic predisposition, such as bronchial asthma. We also previously found that high levels of IgE were detected in the MEE of patients with EOM.⁷ We speculate that IgE may be locally produced in the middle ear mucosa. In chronic rhinosinusitis, massive infiltration of eosinophils is also observed in the sinus mucosa or nasal polyps of some patients. This condition is called eosinophil-dominant nasal polyposis or eosinophilic chronic rhinosinusitis.

Bachert et al⁸ found that *Staphylococcus aureus* enterotoxin is an important factor in causing eosinophilic inflammation in nasal polyps and is characterized by an increased level of IgE antibody against *S aureus* enterotoxin. Recently, certain fungi, such as *Aspergillus* and *Alternaria*, were also found to be responsible for

Reprints: Yukiko Iino, MD, PhD, Department of Otolaryngology, Jichi Medical University Saitama Medical Center, 1-847 Amanuma-cho, Omiya-ku, Saitama 330-8503, Japan; E-mail: yiino@jichi.ac.jp.

Disclosures: Authors have nothing to disclose.

Funding Sources: This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (22591889).

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 cedar, 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.

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 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.

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
<i>Aspergillus</i>	9	0
<i>Alternaria</i>	9	0
<i>Candida</i>	11	0
<i>Mucor</i>	8	0
<i>Penicillium</i>	7	0
<i>Cladosporium</i>	8	0
<i>Staphylococcus aureus</i>		
Enterotoxin A	7	0
Enterotoxin B	5	1
Negative	10	8

Abbreviation: EOM, eosinophilic otitis media.

^aP < .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 cedar 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

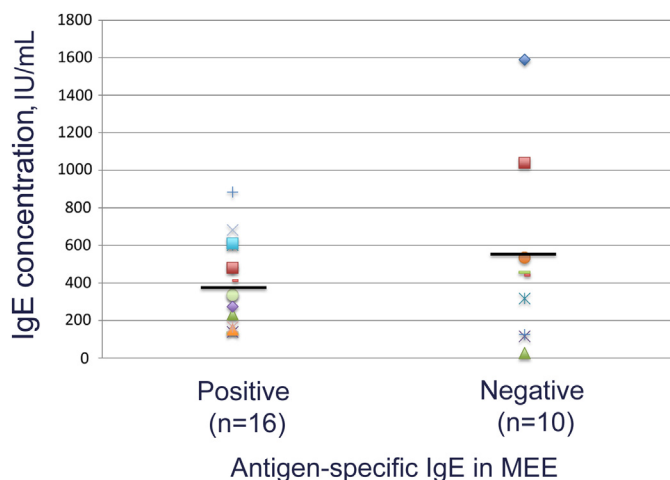


Figure 1. Total serum IgE concentrations in patients with eosinophilic otitis media regarding the presence of 1 or more antigen-specific IgEs in middle ear effusion (MEE).

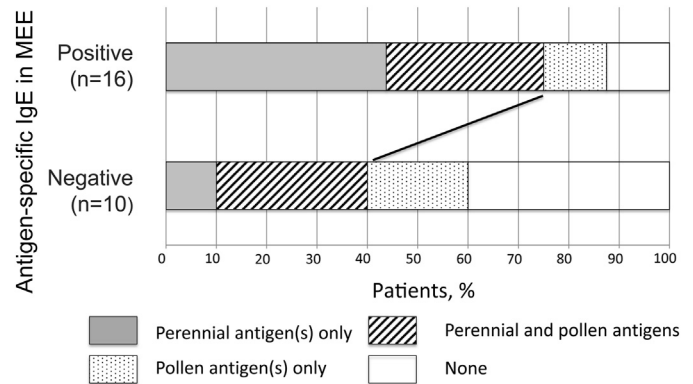


Figure 2. Percentage of patients with perennial or pollen antigen-specific IgE in serum regarding the presence of 1 or more antigen-specific IgEs in middle ear effusion (MEE) (P = .08).

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.

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
<i>Staphylococcus aureus</i>	10	1
Methicillin-resistant <i>S aureus</i>	2	0
<i>Pseudomonas aeruginosa</i>	3	2
<i>Haemophilus influenzae</i>	0	3
<i>Streptococcus pneumoniae</i>	1	0
<i>Candida</i>	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 al⁹ 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 al¹⁹ 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 al²⁵ 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 Larynx*. 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, Cieslak M, Perez-Novoa 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] Ponicaud 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, Agbetle 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 Cauwenberge 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.