

Eosinophilic Inflammation in the Middle Ear Induces Deterioration of Bone-Conduction Hearing Level in Patients With Eosinophilic Otitis Media

Yukiko Iino, Hajime Usubuchi, Kozue Kodama, Hiromi Kanazawa,
Katsumi Takizawa, Takeharu Kanazawa, and Yasushi Ohta

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

Objective: Eosinophilic otitis media (EOM) is characterized by the extensive accumulation of eosinophils in the middle ear mucosa and middle ear effusion and is usually associated with bronchial asthma. Eosinophilic otitis media patients show gradual or sudden deterioration of hearing. In our previous study, we reported that high-tone loss was more frequently found and more severe in EOM patients than in control patients with chronic otitis media. These findings suggest that not only bacterial infection but also eosinophilic inflammation in the middle ear may damage the inner ear. The present study was performed to determine whether eosinophilic inflammation is indeed related to deterioration of bone-conduction hearing level (BCHL).

Patients: Fifty-five ears of 28 patients with EOM associated with bronchial asthma were included in this study. Middle ear effusion (MEE) samples were collected from all the patients, and the concentrations of eosinophilic cationic protein (ECP) and immunoglobulin E (IgE) were measured by fluorescence enzyme immunoassay. The BCHLs at 2 and 4 kHz for the worse-hearing

ear of each patient were correlated with the concentrations of ECP and IgE.

Results: The concentration of IgE in MEE significantly and positively correlated with BCHL at 2 and 4 kHz. The ears with a higher concentration of ECP in MEE also tended to show deterioration of BCHL at 4 kHz. Other clinical risk factors for BCHL deterioration were male sex, long duration of EOM, association with bacterial infection, severe inflammatory changes of the middle ear mucosa, and high serum IgE concentration.

Conclusion: Eosinophilic-inflammation-related substances such as ECP and IgE are closely related to the deterioration of BCHL at high frequencies. Particularly, IgE concentration in MEE is a good indicator of BCHL elevation. We should always pay attention to the hearing acuity of EOM patients with the risk factors. **Key Words:** Bone conduction hearing level—Eosinophil—Eosinophil cationic protein—Eosinophilic otitis media—Immunoglobulin E.

Otol Neurotol 31:100–104, 2010.

Eosinophilic otitis media (EOM) (1) is an intractable disease characterized by the presence of a highly viscous yellow effusion with extensive accumulation of eosinophils in the middle ear. It manifests as otitis media with effusion (OME) when there is no perforation of the eardrum and as chronic otitis media (COM) when there is a persistent perforation of the eardrum because of the spontaneous rupture of the eardrum or myringotomy/insertion of a tympanostomy tube. It mainly occurs in patients with bronchial asthma and is resistant to conventional treatments for OME and COM. One of the most distinct characteristics of EOM is the high incidence of sensorineural hearing loss independent of age. It has also been

reported that in EOM patients, sensorineural hearing loss can be gradual or at times of sudden onset (1–3). In our recent study, high-tone loss was more frequently found and more severe in EOM patients than in COM control patients. At 4 kHz, a significantly greater elevation of bone-conduction hearing level (BCHL) was observed in patients with EOM than in control patients without bronchial asthma (4). We also found that the risk factors for the deterioration of BCHL at 4 kHz were older age, male sex, presence of pathogens in middle ear effusion (MEE), and inflammatory condition of the middle ear mucosa. The cause of sensorineural hearing impairment in EOM remains unclear. However, these findings suggest that bacterial infection and eosinophilic inflammation in the middle ear might exert a synergistic effect on inner ear damage.

The eosinophilic cationic protein (ECP) is a cytotoxic protein derived from eosinophils and is an indicator of eosinophilic inflammation. Immunoglobulin E (IgE) is produced by B cells and plays an important role in Type I

Address correspondence and reprint requests to Yukiko Iino, M.D., Ph.D., 1-847, Amanuma-cho, Omiya-ku, Saitama 330-8503, Japan; E-mail: yiino@jichi.ac.jp

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

allergy, in which the binding between an antigen and IgE induces the activation of mast cells to produce many types of mediator, including cytokines and chemokines, which cause and aggravate eosinophilic inflammation.

In the present study, we measured the concentrations of ECP and IgE in MEE as markers of eosinophilic inflammation (5,6). We further analyzed the correlation between these concentrations and BCHL in EOM patients to determine whether eosinophilic inflammation is indeed related to the deterioration of BCHL.

PATIENTS AND METHODS

Patients

Fifty-five ears of 28 patients with EOM associated with bronchial asthma were included in this study. One patient was unilaterally affected by EOM. They were 17 women and 11 men aged 30 to 76 years old (mean $\pm$ SD, 55.6 ± 10.4 yr). They were diagnosed as having EOM with a highly viscous MEE in which the accumulation of eosinophils was detected histologically or cytologically on the basis of our diagnostic criteria (3). They were all treated with inhaled steroids or by systemic administration of steroids for bronchial asthma. The baseline characteristics of EOM patients are shown in Table 1. As controls, 16 patients with OME not associated with bronchial asthma or allergic predispositions were included. They were 8 women and 8 men aged 28 to 75 years old (mean $\pm$ SD, 53.1 ± 14.6 yr). The difference in the age distributions of both groups was not statistically significant.

Preparation of MEE

Middle ear effusion samples were collected using a Jun-Tym-Tap middle ear fluid aspirator/collector (Xomed, Jacksonville, FL, USA). An effusion sample (50–100 mg) was suspended in 1 ml of saline until the fluid became homogeneous by vortexing vigorously at room temperature. The fluid samples were allowed to stand for 60 to 120 minutes then centrifuged at 1,000 to 1,350 g for 10 min. The resultant supernatant was collected and stored at -80°C until measurement.

Measurement of ECP and IgE Concentrations in MEE and Serum

The concentrations of ECP and IgE in the samples were measured by fluorescence enzyme immunoassay.

TABLE 1. Baseline characteristics of the patients with EOM ($n = 28$)

No. ears	55
Gender (F/M)	17/11
Age, yr ^a	55.6 ± 10.5 (30–78)
Duration of EOM, yr ^a	9.8 ± 6.5 (1–28)
Bacterial culture in MEE/otorrhea	
Pathogen positive	11
Nonpathogen positive	5
Negative	12
Condition of middle ear mucosa	
Grade 1	10
Grade 2	9
Grade 3	9
Serum IgE concentration, U/ml ^a	473 ± 335 (58–1590)
Peripheral blood eosinophil, % ^a	14 ± 11 (2–54)

^aMean $\pm$ standard deviation (range).

EOM indicates eosinophilic otitis media; MEE, middle ear effusion.

Evaluation of Hearing

The air- and bone-conduction hearing levels of the patients with EOM were assessed by pure-tone audiometry. Regarding the audiometric results, the latest results were evaluated. When the hearing threshold of a patient at each frequency was beyond the measurement limit of the audiometer, the measurable level plus 5 dB was defined as the hearing level of the patient at that frequency.

Clinical Risk Factors for BCHL

We analyzed the correlation of BCHLs at 2 and 4 kHz of the worse ear in each patient with the concentration of ECP or IgE in each patient. The worse ear was determined by the sum of BCHLs at 2 and 4 kHz. In addition, we also analyzed the following clinical factors for each patient: sex, age, duration from the onset of otitis media, result of MEE/otorrhea culture for bacteria, condition of the middle ear mucosa, serum IgE concentration, and the percentage of eosinophils in peripheral blood. The mucosal condition was classified into 1 of the 3 categories as previously described (4): Grade 1, nearly normal or slightly edematous; Grade 2, edematous or slightly thickened; and Grade 3, highly thickened or granulated to an extent beyond the position of a normal eardrum. We observed the mucosal condition of the middle ear through a perforation in perforated ears or by making a large incision on the eardrum at the time of MEE collection. Informed consent was obtained from all the patients.

Statistical Analyses

Statistical analyses were performed using the unpaired *t* test, Kendall rank correlation methods, and multiple regression analyses. *p* values of less than 0.05 were considered significant.

RESULTS

Concentration of ECP in MEE and Serum

In the control group, MEE from 9 of 16 patients contained ECP at a concentration less than the detection limit (<2 mg/L). However, none of the MEE samples from the EOM group showed ECP at a concentration less than the detection limit. The serum from 11 patients of the EOM group was also subjected to ECP assay. The concentration of ECP was significantly higher in MEE than in serum in the EOM group ($24.6 \pm 24.6 \times 10^3$ versus 27.5 ± 25.2 mg/L; $p < 0.01$). The concentration of ECP in MEE from the EOM group was significantly higher than that from the control group ($24.6 \pm 24.6 \times 10^3$ versus $40.9 \pm 69.6 \times 10$ mg/L; $p < 0.001$; Fig. 1).

Concentration of IgE in MEE and Serum

All the MEE samples from EOM patients had measurable IgE concentrations (>5 U/ml) compared with less than half (7 of 16) from the controls. Serum samples were obtained from all the patients of the EOM group, and the average concentration of IgE in serum was 437.1 U/ml (standard deviation, 334.7 U/ml; normal range, <173 U/ml). The concentration of IgE was significantly higher in MEE than in serum ($p < 0.05$). The IgE concentration in MEE from the EOM group was significantly higher than that in MEE from the control group ($2,799.7 \pm 5,102.9$ versus 160.1 ± 463.7 U/ml; $p < 0.05$; Fig. 1).

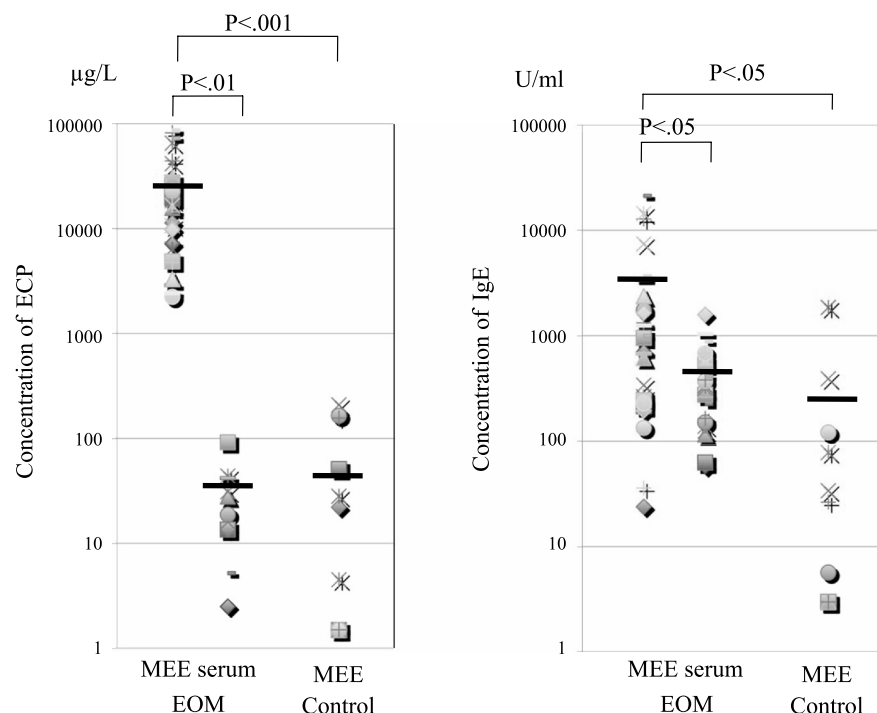


FIG. 1. Concentrations of ECP and IgE in MEE and serum of EOM and control groups.

Evaluation of BCHL in EOM Group

There were 2 EOM patients who showed progressive hearing loss and finally became deaf unilaterally. The average air-conduction hearing levels (ACHLs) and BCHLs at each frequency in 55 ears of the EOM patients are shown in Figure 2. The average ACHL showed a descending type of high-tone loss. The average BCHL also showed high-tone loss, and the BCHLs at 2 and 4 kHz were significantly lower than those at 1 kHz.

Clinical Factors Affecting ECP and IgE Concentrations in MEE

We analyzed the correlation between the clinical factors in each patient and ECP or IgE concentration in the EOM group. The clinical factors, namely, age, sex, results of MEE/otorrhea culture for bacteria, and the condition of the middle ear mucosa, did not affect the concentration of ECP or IgE in MEE. However, IgE concentration in serum positively correlated with that in MEE.

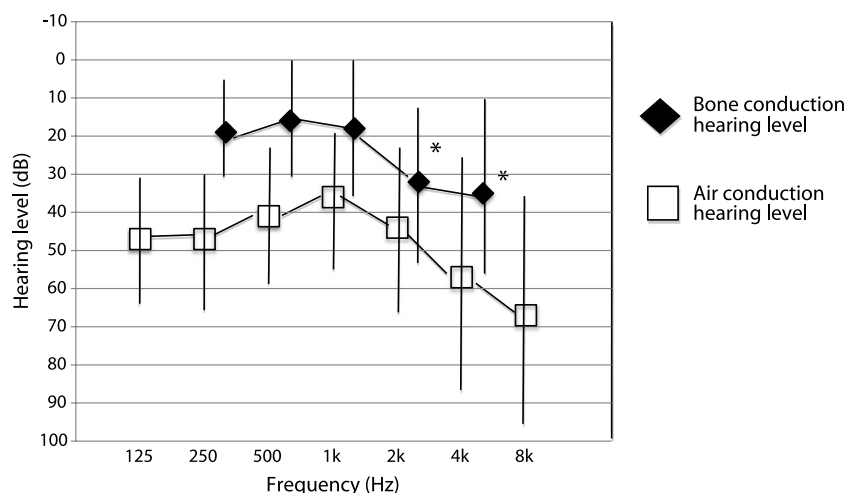


FIG. 2. Average hearing level of patients with eosinophilic otitis media (55 ears). * $p < 0.001$ compared with BCHL at 1 kHz.

TABLE 2. Coefficient of correlation between BCHLs at 2 and 4 kHz in the worse ear and clinical factors of EOM patients (*n* = 28)

Clinical factors	<i>r</i> (k) 2 kHz	<i>r</i> (k) 4 kHz
ECP conc. in MEE	0.1558	0.2197 <i>p</i> = 0.066 ^a
IgE conc. In MEE	0.3338 <i>p</i> < 0.05	0.2996 <i>p</i> < 0.05
Sex	<i>p</i> < 0.01 ^b	<i>p</i> < 0.01 ^b
Age	0.1896	0.2323
Duration of EOM	0.3125 <i>p</i> < 0.05	0.3382 <i>p</i> < 0.05
Bacterial culture in MEE	0.3062 <i>p</i> = 0.056	0.352 <i>p</i> < 0.05
Condition of middle ear mucosa	0.3528 <i>p</i> < 0.05	0.3811 <i>p</i> < 0.05
Serum IgE conc.	0.4005 <i>p</i> < 0.01	0.2996 <i>p</i> < 0.05
Peripheral blood eosinophil, %	0.1975	0.1439

^aAnalyzed by simple correlation coefficient by multiple regression analysis.

^bAnalyzed by *t* test.

EOM indicates eosinophilic otitis media; MEE, middle ear effusion; *r*(k), Kendall rank correlation coefficient.

BCHL and ECP/IgE Concentrations

The results of the statistical analysis of Kendall rank correlation method between the clinical factors and BCHLs at 2 and 4 kHz are shown in Table 2. The concentration of IgE in MEE significantly and positively correlated with the BCHLs at 2 and 4 kHz. The ears with a higher concentration of ECP in MEE tended to show deterioration of BCHL at 4 kHz by multiple regression analysis (simple correlation coefficient, 0.3528; *p* = 0.067). The clinical factors such as sex, duration of EOM, culture results of bacteria in MEE/otorrhea, inflammatory condition of the middle ear mucosa, and serum IgE concentration were also positively correlated with the deterioration of BCHLs at 2 and 4 kHz.

DISCUSSION

In 1947, Koch (7) determined the presence of eosinophils by cytologic examination of MEE from 52 ears of 41 patients of 262 ears with chronic otitis media and reported the following clinical characteristics of these patients: the effusion was highly viscous, and the middle ear mucosa was edematous and thickened. Most of these patients had allergic rhinitis, and its association with bronchial asthma was noted in only 1 patient. After that, Derlacki and Shambaugh (8,9) described the important role of allergy in chronic OME and COM in their articles and called the condition allergic otitis media. However, they did not refer to the deterioration of bone-conduction hearing or the association of bronchial asthma in the patients with allergic otitis media.

Since Tomioka et al. (2) reported the cases of intractable OME and COM patients with bronchial asthma in 1993, similar case reports have been reported and published in

Japan. One of the striking clinical characteristics was the high incidence of gradual or sudden deterioration of hearing. The clinical surveillance of EOM in Japan demonstrated that among 190 EOM patients, approximately one half showed deterioration of BCHL, and 6% became completely deaf (10). In our previous study, 2 (5.3%) of 38 patients became deaf unilaterally after the onset of EOM (11). However, it is generally accepted that ears with COM also show gradual sensorineural hearing loss even if the labyrinthine fistula is not present (12–14). We have shown that high-tone loss was more frequently found and more severe in EOM patients than in age-matched COM control patients (4). Nakagawa et al. (15) also reported that in the case of EOM, BCHLs at 4 and 8 kHz were higher than those at lower frequencies, and the loss rate was approximately 10 times that in the case of COM, which was reported by Cusimano et al. (14). In this study of 55 ears of 28 patients with EOM, a descending type of high-tone loss of ACHL was observed, and BCHLs at 2 and 4 kHz were much lower than those at 1 kHz (Fig. 2) in accordance with the previous reports (4,15). The BCHL at 2 kHz may also be affected by a middle ear component. We monitored constantly the hearing acuity of EOM patients before and after the removal of MEE by myringotomy to observe the changes in BCHL. The BCHL at frequencies including 2 kHz remained the same when adequate treatment of EOM was administered to the patients. Therefore, the deterioration of BCHL at 2 kHz could mainly be caused by inner ear damage.

The cause of deterioration of BCHL in EOM has not been determined. From the results of our previous analysis, bacterial infection and eosinophilic inflammation must contribute to the deterioration of inner ear function (4). In the present study, we measured ECP and IgE concentrations in MEE as markers of eosinophilic inflammation in the middle ear. Significantly higher concentrations of ECP and IgE were detected in MEE than in serum of EOM patients (Fig. 1), indicating that these substances were not derived from the blood but were locally produced in the middle ear. In addition, the MEE from the EOM patients contained significantly higher concentrations of ECP and IgE than that from the control patients (Fig. 1), suggesting that both are certainly important markers of eosinophilic inflammation.

Eosinophils play an essential role in various allergic respiratory diseases such as bronchial asthma and allergic rhinitis. Eosinophils induce various inflammatory responses, including chemotaxis and activation of immunocompetent cells, enhancement of permeability of vessels, and mucus production, by secreting many inflammatory mediators. The eosinophils are also capable of releasing active oxygen species and cytotoxic granular proteins such as ECP to damage tissues. In the present study, we found that the concentration of ECP tended to correlate with BCHL at 4 kHz (Table 2), suggesting that eosinophilic inflammation may be responsible for the inner ear damage.

In Type I allergy, eosinophils also play an important role in the late-phase reactions. In an animal model of bronchial asthma in late-phase reactions, IgE, but not

IgG₁, induces pulmonary inflammation and hyperresponsiveness (16). FcεRI expressed on an eosinophil directly participates in IgE-dependent hypersensitivity reaction to release cytotoxic granular proteins, resulting in tissue damage (17). Therefore, the presence of a high concentration of IgE must aggravate middle ear inflammation. In the present study, we found the significantly positive correlation of the IgE concentration in MEE and BCHLs with 2 and 4 kHz (Table 2), indicating that IgE may aggravate eosinophilic inflammation in the middle ear, thus inducing inner ear damage.

The present study showed that the effects of ECP and IgE in MEE on BCHL were independent of the clinical factors except for serum IgE concentration. High serum IgE concentration can affect the IgE concentration in MEE, although the IgE concentration in MEE was 10 times higher than that in serum. Immunoglobulin E in MEE may not be derived from the serum but locally produced in patients with atopic predisposition.

The other risk factors for the deterioration of BCHL at 2 or 4 kHz were male sex, bacterial infection of the ears, inflammatory condition of the middle ear mucosa, and duration of EOM (Table 2). The former 3 factors were consistent with the findings of our previous report, and the latter factor was consistent with the findings of Nakagawa et al. (15).

The mechanism underlying inner ear damage by bacterial and eosinophilic inflammation is unclear. Eosinophils are much more cytotoxic than neutrophils (18) because eosinophils can generate a higher level of reactive oxygen species than neutrophils (19). The cytotoxic proteins and active oxygen species generated by eosinophils may damage the epithelial layer, including the round window membrane, and lipid mediators released from eosinophils render the membrane permeable. These events allow inflammatory substances such as bacterial toxins and inflammatory cytokines to enter into the inner ear, resulting in inner ear damage. Therefore, we should always focus our attention on ear symptoms and monitor the hearing acuity of EOM patients. We have recently reported that the instillation of triamcinolone acetonide into the middle ear is very effective for controlling otorrhea or MEE in EOM patients (20). Indeed, since we started the treatment using triamcinolone acetonide, we have not encountered patients who became deaf or showed progressive hearing loss.

In conclusion, the severity of eosinophilic inflammation in the middle ear is closely related to the deterioration of BCHL in EOM, and IgE concentration in MEE may be a good indicator of BCHL elevation. It is very important to inform high-risk patients that without adequate treatment, progressive or sudden onset of severe hearing loss may occur.

REFERENCES

1. 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: CIC Edizioni Internazionali, 1997:851–3.
2. 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/New York: Kugler Publications, 1993:183–6.
3. Nagamine H, Iino Y, Kojima C, Miyazawa T, Iida T. Clinical characteristics of so-called eosinophilic otitis media. *Auris Nasus Laryx* 2002;29:19–28.
4. Iino Y, Usubuchi H, Kodama K, et al. Bone conduction hearing level in patients with eosinophilic otitis media associated with bronchial asthma. *Otol Neurotol* 2008;29:949–52.
5. Barck C, Lundahl J, Halldén G, Bylin G. Total eosinophil cationic protein levels in induced sputum as a marker of changes in eosinophilic inflammation in a patient with allergic asthma. *Ann Allergy Asthma Immunol* 2005;95:86–92.
6. Zeller S, Glaser AG, Vilhelmsson M, Rhyner C, Cramer R. Immunoglobulin-E-mediated reactivity to self antigens: a controversial issue. *Int Arch Allergy Immunol* 2008;145:87–93.
7. Koch H. Allergical investigations of chronic otitis. *Acta Otolaryngol* 1947;(Suppl 62):1–201.
8. Derlacki EL. Aural manifestations of allergy. *Ann Otol Rhinol Laryngol* 1952;61:179–8.
9. Derlacki EL, Shambaugh GE Jr. Allergic management of some common ear conditions. *Trans Am Acad Ophthalmol Otolaryngol* 1953;57:304–12.
10. Suzuki H, Matsutani S, Kawase T, et al. Epidemiologic surveillance of “eosinophilic otitis media” in Japan. *Otol Jpn* 2004;14:112–7. (In Japanese).
11. Iino Y. Eosinophilic otitis media: a new middle ear disease entity. *Curr Allergy Asthma Rep* 2008;8:525–30.
12. El-Sayed Y. Bone conduction impairment in uncomplicated chronic suppurative otitis media. *Am J Otolaryngol* 1998;19:149–53.
13. Blakley BW, Kim S. Does chronic otitis media cause sensorineural hearing loss? *J Otolaryngol* 1998;27:17–20.
14. Cusimano F, Cocita VC, D’Amico A. Sensorineural hearing loss in chronic otitis media. *J Laryngol Otol* 1989;103:158–63.
15. Nakagawa T, Matsubar A, Shiratsuchi H, et al. Intractable otitis media with eosinophils; importance of diagnosis and validity of treatment for hearing preservation. *ORL J Otorhinolaryngol Relat Spec* 2006;68:118–22.
16. Itami, DM, Latinne D, Bazin H, et al. Immunoglobulin E is not required for but enhances airway inflammation and hyperresponsiveness. *Allergy* 2003;58:1117–24.
17. Capron M. Eosinophils: receptors and mediators in hypersensitivity. *Clin Exp Allergy* 1989;19:3–8.
18. Robers RL, Ank BJ, Stiehm ER. Human eosinophils are more toxic than neutrophils in antibody-independent killing. *J Allergy Clin Immunol* 1991;6:1105–15.
19. Shult PA, Graziano FM, Wallow IH, Busse WW. Comparison of superoxide generation and luminal-dependent chemiluminescence with eosinophils and neutrophils from normal individuals. *J Lab Clin Med* 1985;106:638–45.
20. Iino Y, Nagamine H, Kakizaki K, et al. Effectiveness of instillation of triamcinolone acetonide into middle ear for eosinophilic otitis media associated with bronchial asthma. *Ann Allergy Asthma Immunol* 2006;97:761–6.