ophil count was >350/microscopic field (400× magnification in hematoxylin and eosin stain) using the top five fields in terms of rich eosinophilic infiltration. The non-ECRS included nine patients in whom the eosinophil count was <100/microscopic field (400× magnification) in any of the fields. Five cases in which normal mucosal membranes of the sphenoid sinus were removed during surgery for pituitary adenoma were used as controls. All patients gave their written informed consent, and the study was approved by the Ethics Committee of Juntendo University School of Medicine.

Immunostaining Analysis for CD68 (Marker of Macrophages), IL-17A, Monocyte Chemotactic Protein 1, Neutrophil Elastase (Marker of Neutrophils), IL-8, and MUC5AC

The nasal polyps were fixed in 10% formalin, embedded in paraffin wax, processed routinely, and then prepared as routine semithin sections (3.5 μm).

Macrophages were observed using mouse anti-human CD68 1:3 (Dako, Tokyo, Japan) and sections treated with immunoblock served as the negative control. The expressions of IL-17A and monocyte chemotactic protein (MCP) 1 were examined by rabbit anti-mouse IL-17A antibody 1:50 (Santa Cruz Biotechnology, Santa Cruz, CA) and anti–MCP-1 antibody 1:200 (R & D, Minneapolis, MN) with rabbit serving IgG1 as the negative control.

Neutrophils were observed using mouse anti-human neutrophil elastase 1:100 (Dako) with mouse IgG1 as the negative control. The expression of IL-8 was examined by goat anti-mouse IL-8 antibody 1:5 (R & D) with immunoblock as the negative control.

MUC5AC was observed using mouse anti-human MUC5AC 1:3 (Bio Science for the World, Santa Barbara, CA); sections treated with mouse IgG1 served as the negative control. The sections were stained with the Ventana /VEW DAB Detection kit using a Ventana automated stainer (Ventana Japan KK, Yokohama, Japan).

The numbers of cells positive for CD68, IL-17A, MUC5AC, MCP-1, and IL-8 and elastase were assessed by the mean of the top three fields in terms of the richness of their infiltration (400× magnification).

Statistical Analyses

The data were expressed as mean ± SD. Statistical analyses were performed using StatMateIII for Windows. Statistical analyses were evaluated using Pearson's correlation coefficient and Student's *t*-test. A value of $p < 0.05$ was considered significant.

RESULTS

We identified macrophages using CD68. Most of the $CD68^+$ macrophages (47 ± 23/a field) had infiltrated into the subepithelia of ECRS (Fig. 1), whereas non-ECRS showed few macrophages. A significantly larger mean number of $CD68^+$ macrophages was observed in ECRS (47 ± 23/a field) compared to non-ECRS (20 ± 11/a field) and the control (17 ± 7/a field) group ($p < 0.001$; Fig. 2). There was no significant difference in the mean number of macrophages between non-ECRS and the control group.

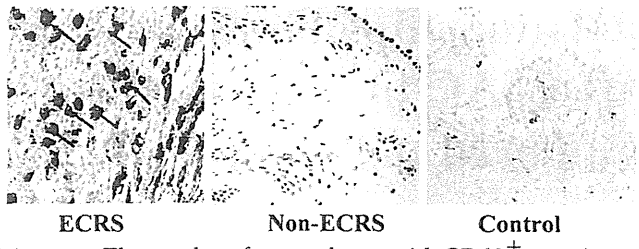

Figure 1. The number of macrophages with $CD68^+$ reactions of eosinophilic chronic rhinosinusitis (ECRS); arrows, right) was significantly more than in non-ECRS (middle) and control (left).

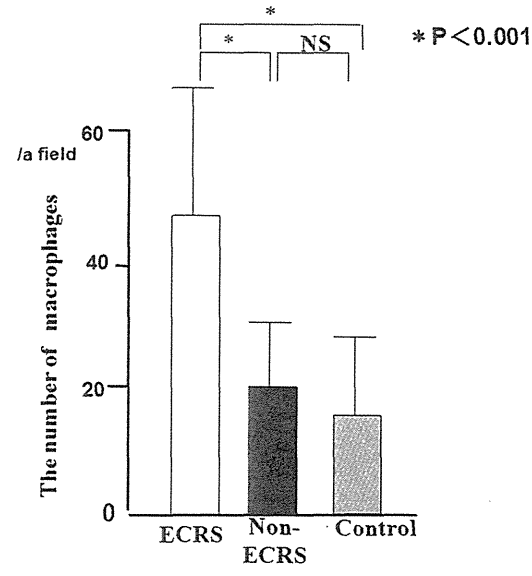

Figure 2. The number of macrophages with CD68 expression in the subepithelia.

IL-$17A^+$ inflammatory cells were observed in CRS (Fig. 3). A significantly larger mean number of IL-$17A^+$ cells was seen in ECRS (48 ± 18/a field) compared with non-ECRS (14 ± 13/a field) and the control group (9 ± 7/a field; Fig. 4). There was no significant difference in the mean number of IL-$17A^+$ cells between non-ECRS and the control group. A significant correlation was recognized in all cases between CD68 and IL-$17A^+$ cells ($p < 0.01$; Fig. 5).

The mean percentages of $MUC5AC^+$ epithelial cells reaction in ECRS (26.5 ± 21.1%/a field) were significantly greater than in non-ECRS (9.2 ± 7.5/a field) and the control group (7.0 ± 5.1/a field; Fig. 6;

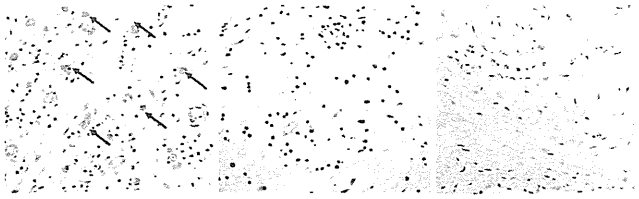

Figure 3. Immunohistochemical demonstration of IL-17A in chronic rhinosinusitis (CRS). The number of cells with IL-17A$^+$ reactions of eosinophilic CRS (ECRS; arrows, right) was significantly more than in non-ECRS (middle) and controls (left).

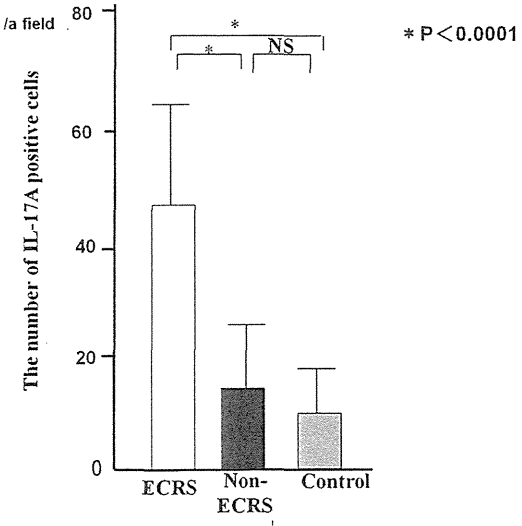

Figure 4. The number of IL-17A$^+$ cells in chronic rhinosinusitis (CRS).

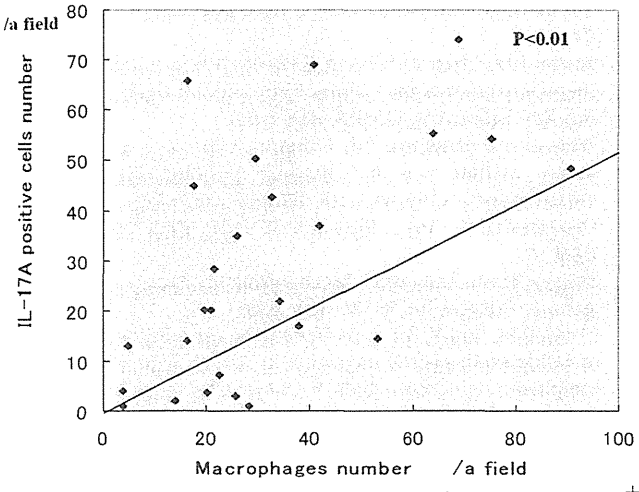

Figure 5. Relationship between the number of IL17A and CD68$^+$ cells.

$p < 0.05$). There was a significant correlation between MUC5AC and IL-17A$^+$ cells in all cases (Fig. 7; $p < 0.05$)).

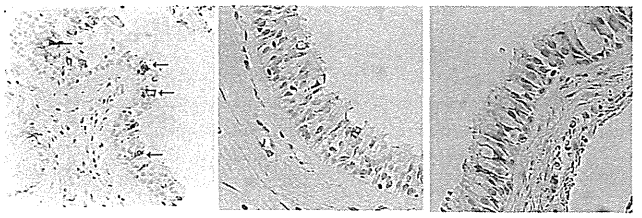

Figure 6. The number of MUC5AC positive cells in ECRS (arrows, left) was significantly greater than in non-ECRS(middle) and control (right).

The mean number of MCP-1$^+$ cells in ECRS (20 ± 31/ field) was more than twice that in non-ECRS (10 ± 5). However, the difference between ECRS and non-ECRS or the control group was not significant. No correlation between CD68 and MCP-1$^+$ cells was observed.

The mean number of neutrophils (positive for neutrophil elastase) did not show significant differences between ECRS (10 ± 14/a field) and non-ECRS (14 ± 11/a field). The mean number of IL-8$^+$ cells in ECRS (11 ± 17/a field) was greater than that in non-ECRS (6 ± 5/a field), but not significantly.

DISCUSSION

Poston[2] reported that, in asthmatic patients, the total number of macrophages infiltrating the airway mucosa was increased and suggested that lung macrophages may have a central role in the chronic immune-mediated inflammatory response seen in the airway mucosa of asthmatic patients. Previously, we examined whether severe epithelial damage would be associated with infiltrating eosinophils in ECRS.[12] Our results showed that the number of CD68$^+$ macrophages in the subepithelia was significantly greater in ECRS than in non-ECRS. Macrophages as well as eosinophils are able to produce many cytotoxic agents such as oxidants, *e.g.*,[13,14] and metalloproteinases.[6] Based on the aforementione reports and our results, the remodeling in ECRS may be associated with infiltrating macrophages as well as eosinophils. Concerning the infiltrating macrophages in our experiment, we suggest two theories as follows. First, macrophages were phagocytotic for wastes in nasal mucosa because of inflammation as a native function. Second, eosinophils might release some chemokines for macrophages.

The present study was consistent with our previous report,[15] in which a significant increase in the mean number of IL-17A$^+$ cells in ECRS was observed in comparison with non-ECRS. The expression of IL-17A$^+$ cells indicated inflammatory cells including both eosinophils and lymphocytes. We revealed a significant relationship between eosinophils and IL-17A$^+$ cells and suggested that eosinophils could

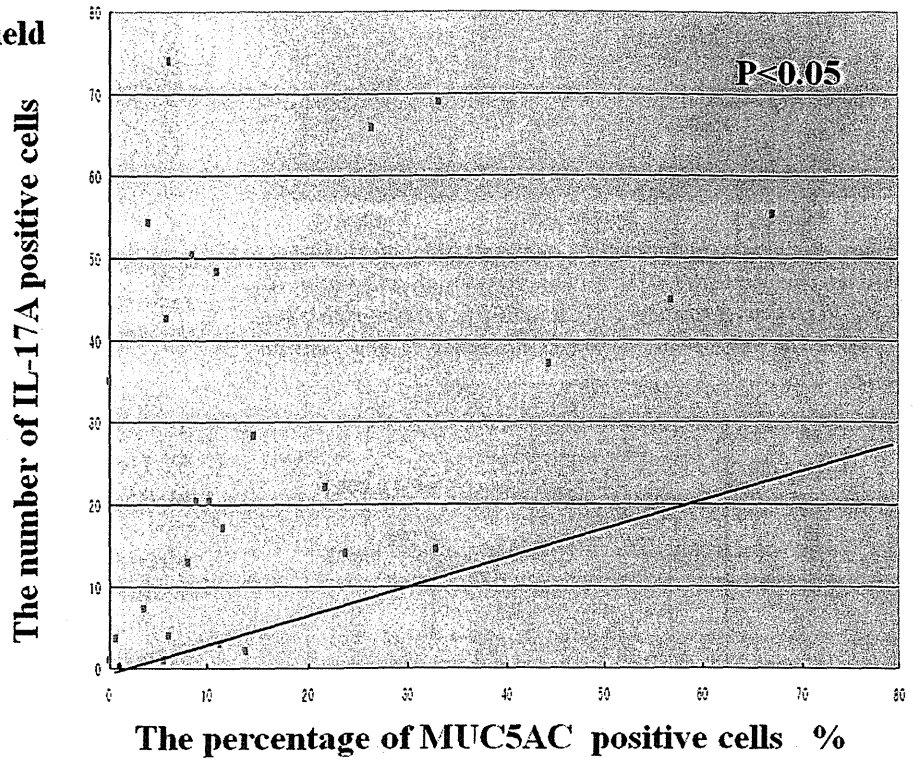

Figure 7. Relationship between the number of MUC5AC and IL-17A$^+$ cells.

product IL-17A. Makihara[8] reported that IL-17A expression in CRS could be found in eosinophils, macrophages, and lymphocytes by double staining. Moreover, our study could show a significant correlation between CD68 and IL-17A$^+$ cells in all cases. Thus, macrophages as well as eosinophils are a possible source of IL-17A in ECRS. ECRS is characterized by enriched amounts of mucin-producing cells and eosinophilic mucus secretion. The key factor in the mucus production in ECRS is still unknown. In the present study, IL-17A$^+$ cells were significantly correlated with MUC5AC$^+$ cells, which suggest that IL-17A plays a critical role in mucus secretion. Chen[9] reported that IL-17A could induce mucin gene expression (such as MUC5AC) in the airways and that there was a significant correlation between MUC5AC and IL-17A$^+$ cells. Therefore, IL-17A in the subepithelia is likely to stimulate the MUC5AC expression of epithelia in ECRS as well as airways.

IL-8 can induce neutrophilic inflammation in both upper and lower airways.[16] Silvestri[17] reported that patients with severe asthma showed significantly higher amounts of IL-8 than mild–moderate asthmatic patients or controls, and increase the numbers of neutrophils. Yoshihara[18] reported that epithelial damage is caused by enhanced IL-8 and the activity of neutrophils during acute exacerbations of pediatric asthma. Cundall[5] described that neutrophil-derived MMP-9 was increased in severe asthma. However, neither neutrophils nor IL-8 showed significant differences between ECRS and non-ECRS in our study.

In conclusion, IL-17A, macrophages and MUC5AC are the key factors in the processes of ECRS and would be more significant than neutrophils or IL8.

REFERENCES

1. Seminario MC, and Geich GJ. The role of eosinophils in the pathogenesis of asthma. Curr Opin Immuno 6:860–864, 1994.
2. Poston RN, Chanez P, Lacoste JY, et al. Immunohistochemical characterization of the cellular infiltration in asthmatic bronchi. Am Rev Respir Dis 145:918–921, 1992.
3. Wenzel SE, Schwartz LB, Langmack E, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 160:1001–1008, 1999.
4. Togaya E, and Tamaoki J. Mechanisms of airway remodeling in asthma. Allergol Int 56:331–340, 2007.
5. Cundall M, Sun Y, Miranda C, et al. Neutrophil-derived matrix metalloproteinase-9 is increased in severe asthma and poorly inhibited by glucocorticoids. J Allergy Clin Immunol 112:1064–1071, 2003.
6. Martney JM, Gustafson CE, Bowler RP, et al. Thromboxane receptor signaling is required for fibronectin-induced matrix metalloproteinase-9 production by human and murine macrophages and is attenuated by the Arhgef1 molecule. J Biol Chem 286:44521–44531, 2011
8. Makihara S, Okano M, Fujiwara T, et al. Regulation and characterization of IL-17A expression in patients with chronic rhinosinusitis and its relationship with eosinophilic inflammation. J Allergy Clin Immunol 126:397–400, 400.e1–e11, 2010.

9. Chen Y, Thai P, Zhao YH, et al. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. J Biol Chem 278:17036–17043, 2003.
10. Vento SI, Ertama LO, Hytönen ML, et al. Nasal polyposis: Clinical course during 20 years. Ann Allergy Asthma Immunol 85:209–214, 2000.
11. Fokkens W, Lund V, Bachert C, et al. EAACI position paper on rhinosinusitis and nasal polyps executive summary. Allergy 60:583–601, 2005.
12. Saito T, Kusunoki T, Yao T, et al. Relationship between epithelia 1 damage or basement membrane thickness and eosinophilic infiltration polyps with chronic rhinosinusitis. Rhinology 47: 275–279, 2009.
13. Vendrow AE, Hakim ZS, Madamanchi NR, et al. Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. Ateriosclerosis Thromb Vasc Biol 27:2714–2721, 2007.
14. Ezeamuzie CI, and Taslim N. Reactive oxygen species mediate phorbol ester-stimulated cAMP response in human eosinophils. Eur J Pharmacol 543:174–180, 2006.
15. Saito T, Kusunoki T, Yao T, et al. Role of interleukin-17A in the eosinophil accumulation and mucosal remodeling in chronic rhinosinusitis with nasal polyps associated with asthma. Int Arch Allergy Immunol 151:8–16, 2010.
16. Suzuki H, and Ikeda K. Mode of action of long-term low-dose macrolide therapy for chronic sinusitis in the light of neutrophil recruitment. Curr Drug Targets Inflamm Allergy 1:117–126, 2002.
17. Silvestri M, Bontempelli M, Giacomelli M, et al. High serum levels of tumor necrosis factor-alpha and interleukin-8 in severe asthma: Makers of systemic inflammation. Clin Exp Allergy 36:1373–1381, 2006.
18. Yoshihara S, Yamada Y, Abe T, et al. Association of epithelial and signs of neutrophil mobilization in the airways during acute exacerbations of paediatric asthma. Clin Exp Immunol 144:212–216, 2006. □

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Heme oxygenase-1 expression in chronic rhinosinusitis with eosinophilic infiltration

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ABSTRACT

Objectives: Chronic rhinosinusitis (CRS) with eosinophilic infiltration is a type of intractable rhinosinusitis often associated with asthma. The oxidants are well known to induce aggravate asthma. Heme oxygenase-1 (HO-1), a cytoprotective enzyme against oxidant, has been extensively studied in airway diseases. However, no study that observed HO-1 in both epithelial and subepithelial tissues of CRS has been reported.

Methods: Part of each specimen derived from the nasal polyps of CRS with and without eosinophilic infiltration was promptly fixed for hematoxylin–eosin staining and immunohistochemical analysis for HO-1 and macrophages.

Results: We found that the expression of HO-1 in the epithelial layers of CRS without eosinophilic infiltration was significantly enhanced as compared with that of CRS with eosinophilic infiltration. On the other hand, the number of macrophages with HO-1 positive reactions was significantly greater in CRS with eosinophilic infiltration compared with CRS without eosinophilic infiltration.

Conclusions: Our study suggests that both a reduction of HO-1 expression in epithelial cells and an increase of infiltration of macrophages positive for HO-1 are related to the epithelial damage of CRS with eosinophilic infiltration.

1. Introduction

Heme oxygenase (HO) is an enzyme with many functions that can catabolize heme to produce carbon monoxide, free iron, and biliverdin. Biliverdin is rapidly changed to the antioxidant bilirubin by the enzyme biliverdin reductase, and any free iron is sequestrated by ferritin [1]. Three kinds of HO have been identified. HO-1 is a 32-kDa protein that can be induced in cells by a variety of agents, including oxidative stress, heavy metals, ultraviolet light, and heme and its derivatives [2]. Chronic inflammatory lung diseases are associated with increased production of oxidants. Induction of HO-1 by reactive oxygen species is a cytoprotective mechanism against oxidative stress [3].

Macrolides are well known to contribute to the amelioration of chronic rhinosinusitis (CRS), characterized as a neutrophil-dominant type [4,5]. On the other hand, we recently encountered intractable rhinosinusitis with resistance to macrolides, which is dependent upon eosinophilic infiltration. CRS with eosinophilic infiltration is an intractable disease closely related to the pathology of asthma [6]. Although oxidants are known to aggravate asthma [7], HO-1 works as an antioxidant [8]. Elhini et al. [9] reported that HO-1 was upregulated in the nasal mucosa with allergic rhinitis. However, there has been no report concerning HO-1 in CRS with eosinophilic infiltration. Therefore, we studied HO-1 by using nasal polyps on both CRS with and without eosinophilic infiltration. Moreover, we describe the relationships between HO-1 and macrophages. Our study will be a helpful key to clarify the processes of intractable diseases such as CRS with eosinophilic infiltration.

2. Materials and methods

2.1. Materials

We examined 17 patients with CRS with nasal polyps who were diagnosed based on the criteria of the European position paper [10], that is, they had two or more symptoms one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), +facial pain/pressure, +reduction or loss of smell; and either endoscopic signs of polyps and/or mucopurulent discharge primarily from middle meatus, and/or oedema/mucosal obstruction primarily in middle meatus, and/or

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Table 1
The characteristics of patients.

	Patient	Age (year)	Sex	Asthma	Eosinophils in serum		Eosinophils in tissue
					Number (μl)	%	Number (/field)
Group 1	1	26	F	–	875	17.5	1161
	2	55	M	–	543	9.7	518
	3	55	F	+	497	12.7	752
	4	59	F	+	638	11.6	427
	5	43	M	+	691	12.8	519
	6	41	M	–	809	10.5	584
	7	28	F	+	1518	16.5	576
	8	29	F	–	685	10.7	386
Group 2	9	49	M	–	72	1.1	72
	10	49	M	–	342	5.9	52
	11	51	M	–	342	5.9	98
	12	57	F	–	422	6.8	92
	13	60	M	–	76	0.8	43
	14	32	M	–	28	0.5	37
	15	21	F	–	9	0.1	4
	16	48	F	–	29	0.9	25
	17	65	M	–	228	4.3	21
Control	18	32	F	–	9	0.4	1
	19	57	M	–	10	0.5	3
	20						5
	21						6
	22	68	M	–	106	0.9	7
	23	41	F	–	99	1.6	8
	24	59	M	–	390	6.1	10

Group 1: chronic rhinosinusitis with eosinophilic infiltration; Group 2: chronic rhinosinusitis without eosinophilic infiltration; open columns are unknown data.

computed tomographic changes showing mucosal changes within ostiomeatal complex and/or sinuses. None of the patients were treated with systemic corticosteroids or other immune-modulating drugs.

The above patients were classified into 2 groups (Table 1). The CRS with eosinophilic infiltration group included 8 patients in which the eosinophil count of the nasal polyp was more than 350 per microscopic field (400× magnification) using the top 5 fields rich in eosinophilic infiltration, or more than 441/μl eosinophils in serum. The CRS without eosinophilic infiltration group included 9 patients in which the eosinophil count of the nasal polyp was less than 200 per microscopic field (400× magnification) in any of the fields or less than 440/μl eosinophils in serum. Seven cases in which normal mucosal membranes of the sphenoid sinus were removed during surgery for pituitary adenoma were used as controls. All patients gave their written informed consent, and the study was approved by the Ethics Committee of Juntendo University School of Medicine.

2.2. Sampling of tissue specimens

Surgically removed human nasal polyps located in the middle meatus were obtained from the patients with CRS. Control samples were obtained from normal mucosal membranes of the sphenoid sinus removed during surgery for pituitary adenoma. The samples were fixed in 10% formalin, embedded in paraffin wax, processed routinely and stained with hematoxylin–eosin.

2.3. Double immunostaining of HO-1 and MBP

The nasal polyps were fixed in 10% formalin, embedded in paraffin wax, processed routinely and then prepared as routine semi-thin sections (3.5 μm). Paraffin sections for double-immunostaining were deparaffinized, rehydrated, and incubated with 0.1% trypsin solution at 37 °C for 30 min. After rinsing, the sections were autoclaved at 121 °C for 10 min in 10 mM citrate buffer pH 6.0. After rinsing, the sections were stained following a sequential method using an iVIEW DAB detection kit and ultra VIEW Universal Alkaline Phosphatase Red kit (Ventana), with a Roche automated stainer Nexes IHC (Ventana) according to the manufacturer's instructions. Primary antibodies were used by mouse anti-human Eosinophil Major Basic Protein (MBP) (abcam, Japan) and anti-Heme Oxygenase-1 antibody (Assay Designs, Ann Arbor MI). The sections were incubated at 37 °C for 32 min with MBP antibody (1:3) and anti-Heme Oxygenase-1 antibody (1:3). Secondary antibodies of MBP and HO-1 were reacted at 37 °C for 8 min and 12 min. The immunohistochemical experiments were controlled by incubation of the tissue with an isotype-matched control mouse IgG1 (DaKo) for MBP and HO-1 at the same concentrations as the primary antibodies. The sections were counterstained with hematoxylin.

2.4. Double immunostaining of HO-1 and CD68

Paraffin sections for double-immunostaining were deparaffinized, rehydrated, and autoclaved at 121 °C for 10 min in 10 mM citrate buffer pH 6.0. Endogenous peroxidase was blocked with inhibitor-D 3% H_2O_2 (Ventana, AZ) for 4 min at 37 °C. After rinsing, the sections were stained following a sequential method using an iVIEW DAB detection kit and ultra VIEW Universal Alkaline Phosphatase Red kit (Ventana) with a Roche automated stainer Nexes IHC (Ventana) according to the manufacturer's instructions. The primary antibodies were mouse anti-human Heme Oxygenase-1 antibody (Assay Designs, Ann Arbor MI) and mouse anti-human CD68 (Dako, Glostrup Copenhagen). The sections were incubated at 37 °C for 32 min with anti-Heme Oxygenase-1 antibody (1:1000) and anti-CD68 antibody (1:100). The secondary antibodies of HO-1 and CD68 were reacted at 37 °C for 8 min and 12 min. The immunohistochemical experiments were controlled by incubation of the tissue with an isotype-matched control mouse IgG2b (DaKo) for HO-1 or mouse IgG3 (R&D System) for CD 68 at the same concentrations as those of the primary antibodies. The sections were counterstained with hematoxylin.

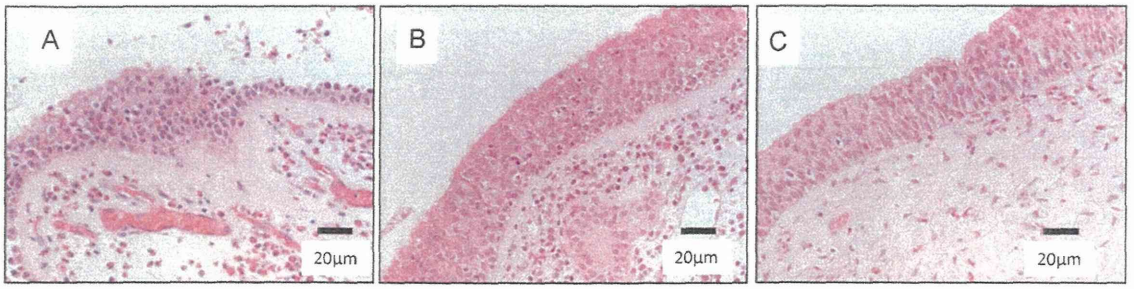

Fig. 1. Hematoxylin–eosin staining of nasal polyps. CRS with eosinophilic infiltration (A) and without eosinophilic infiltration (B) show both many epithelial and goblet cells. Epithelial detachment with infiltrating eosinophils is found in CRS with eosinophilic infiltration (A), but not in CRS without eosinophilic infiltration (B). Some proliferation of epithelial and goblet cells is observed in control mucosa (C).

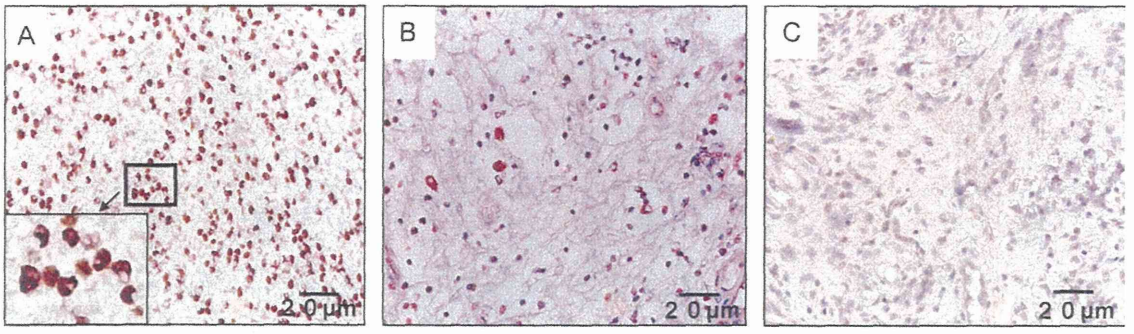

Fig. 2. Double immunostaining of HO-1 and MBP in the subepithelial layer of nasal polyps. The cells positive for both HO-1 MBP are observed in both CRS with eosinophilic infiltration (A) and CRS without eosinophilic infiltration (B). C shows control by isotype-matched control mouse IgG1 (Dako) for HO-1 and MBP. High power – view of the square in (A) indicates MBP positive cells with positive reaction for HO-1. Brown color = MBP; red color = HO-1.

Eosinophils and cells with positive reactions for HO-1, CD 68 or MBP were counted in the top 5 fields that were rich in infiltration with eosinophils and macrophages, and the mean number per a field was calculated. On counting process of the HO-1 positive rate of epithelial cells, we noticed only epithelial cells except for the other cells (e.g. goblet cells). Statistical analysis was evaluated by Mann–Whitney *U* method. A p value less than 0.05 was considered as significant.

3. Results

CRS with eosinophilic infiltration was characterized by epithelial detachment and infiltration with a considerable number of eosinophils with many epithelial and goblet cells (Fig. 1A). On the other hand, in CRS without eosinophilic infiltration, the epithelial layers were composed of many epithelial and goblet cells with little infiltration of eosinophils (Fig. 1B). In the control group, epithelial layers showed some epithelial cells without goblet cells or epithelial sloughing. Subepithelial layers showed none or few inflammatory cells and fibroblasts (Fig. 1C).

Double immunostaining of HO-1 and MBP was examined in subepithelial layers of CRS with eosinophilic infiltration (Fig. 2A) and without eosinophilic infiltration (Fig. 2B). The majority of the HO-1 positive cells were found to be infiltrating eosinophils with MBP positive reaction in both CRS with and without eosinophilic infiltration. A significant increase in number (531/a field) of eosinophils with HO-1 positive reaction in CRS with eosinophilic infiltration was observed in comparison with CRS without eosinophilic infiltration (79/a field) and controls (Fig. 3). However, there was no significant difference in the percentage of eosinophils with HO-1 positive reaction between CRS with eosinophilic infiltration (97.9%, 4196/4284) and CRS without eosinophilic infiltration (84.7%, 552/652). The control group showed a nominal number of HO-1 positive eosinophils as compared to CRS with and without eosinophilic infiltration.

Since a previous study demonstrated that the macrophages in the nasal mucosa of allergic rhinitis expressed HO-1 [9], we

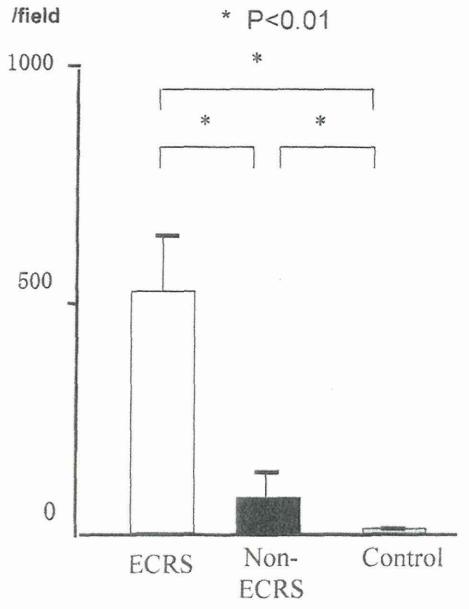

Fig. 3. The number of MBP positive cells with the expression of HO-1 in subepithelial layer. The number of MBP positive cells with the expression of HO-1 in CRS with eosinophilic infiltration was significantly greater than in CRS without eosinophilic infiltration. The control mucosa showed the few infiltrating eosinophils expressing HO-1 as compared with CRS with and without eosinophilic infiltration. $^{*}p < 0.01$.

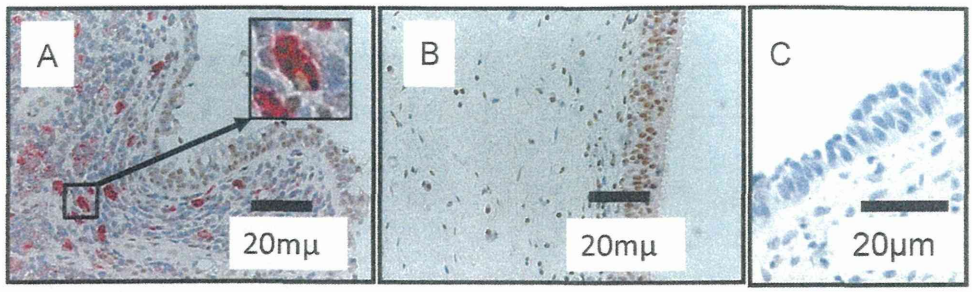

Fig. 4. Double immunostaining of HO-1 and CD68 in the subepithelial layer of nasal polyps in CRS with eosinophilic infiltration (A) and without eosinophilic infiltration (B). C shows control by isotype-matched control mouse IgG2b for HO-1 or mouse IgG3 for CD68. High power-view of the square in (A) indicates CD68 positive cells with positive reaction for HO-1. Brown color = HO-1; red color = CD68.

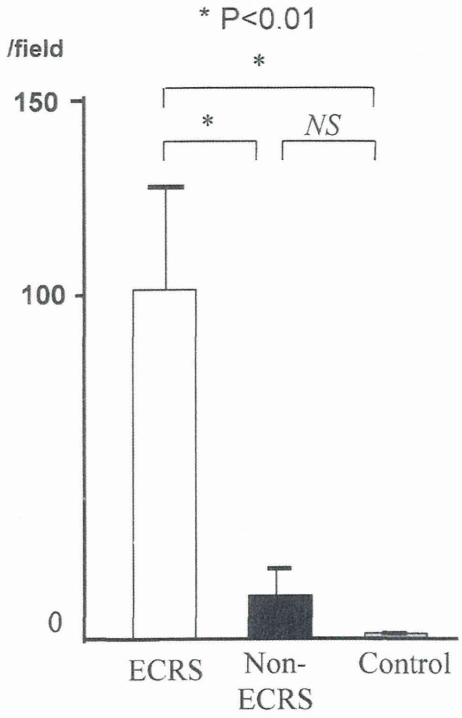

Fig. 5. The number of CD68 positive cells with the expression of HO-1 in subepithelial layer. The number of CD68 positive cells with the expression of HO-1 in CRS with eosinophilic infiltration is significantly greater than in CRS without eosinophilic infiltration and controls. *$p < 0.01$; NS: not significant.

identified macrophages using CD68. A moderate number of CD68 positive macrophages had infiltrated in the subepithelial layer of CRS with eosinophilic infiltration (Fig. 4A), whereas CRS without eosinophilic infiltration showed few macrophages (Fig. 4B). Most of the macrophages in both groups expressed HO-1. Significantly greater numbers (104/a field) of HO-1 positive macrophages were observed in CRS with eosinophilic infiltration compared with CRS (19/a field) without eosinophilic infiltration and controls (Fig. 5). However, there was no significant difference in the percentage of macrophages that were HO-1 positive between CRS with eosinophilic infiltration (98.0%) and CRS without eosinophilic infiltration (95.0%).

Next, the immunoreactivity of HO-1 was observed in both epithelial cells and macrophages infiltrated in the epithelial layer. Although a considerable number of epithelial cells in CRS with eosinophilic infiltration expressed HO-1 (Fig. 6A), CRS without eosinophilic infiltration showed the positive reaction of HO-1 in most of the epithelial cells (Fig. 6B). The HO-1 positive macrophages infiltrated in the epithelial layer in CRS with eosinophilic infiltration (Fig. 6A). In the control, little reaction of HO-1 was observed in the epithelial layer (Fig. 6C). The rate of HO-1 positive epithelial cells in both CRS with and without eosinophilic infiltration was significantly increased as compared with that of controls. The HO-1 positive rate of epithelial cells in CRS without eosinophilic infiltration was significantly greater than that in CRS with eosinophilic infiltration (Fig. 7). In contrast, the number of HO-positive macrophages in the epithelial layer was significantly increased in CRS with eosinophilic infiltration (7/a field) compared to CRS without eosinophilic infiltration (1/a field). However, there was no significant difference in the percentage of macrophages that were HO-1 positive between

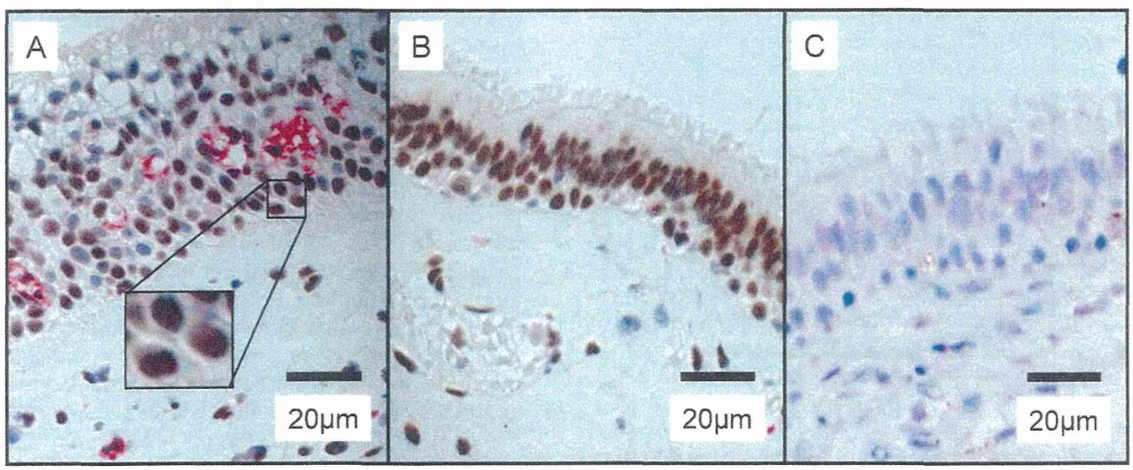

Fig. 6. Double immunostaining of HO-1 and CD68 in the epithelial layer of nasal polyps. Epithelial cells are positive for HO-1 in CRS with eosinophilic infiltration (A) and without eosinophilic infiltration (B). High power-view of the square in (A) indicates the cytoplasm of the epithelial cell stained with HO-1 as positive control whereas control sinus mucosa (C) shows little immunoreaction for HO-1. Brown color = HO-1; red color = CD68.

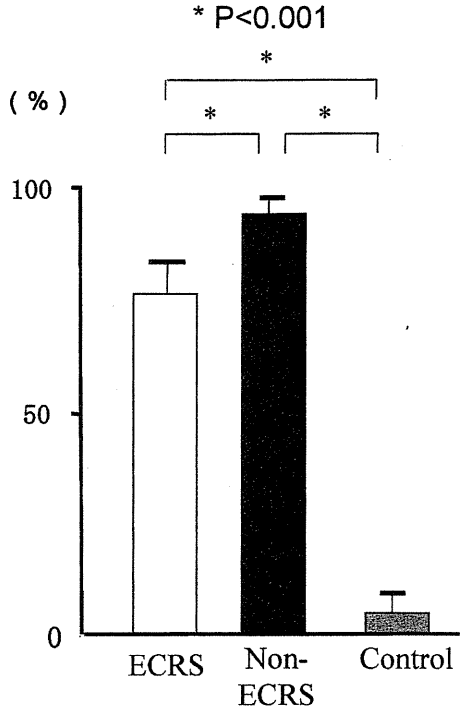

Fig. 7. HO-1 positive rate of epithelial cells. The rate of HO-1 positive epithelial cells in CRS without eosinophilic infiltration is significantly greater than that in CRS with eosinophilic infiltration. Control mucosa shows a significant reduction of the rate as compared with both CRS with and without eosinophilic infiltration. *$p < 0.001$.

CRS with eosinophilic infiltration (100%) and CRS without eosinophilic infiltration (75%). There was also no difference in the percentage of HO-1 positive macrophages between CRS without eosinophilic infiltration and the control group (Fig. 8).

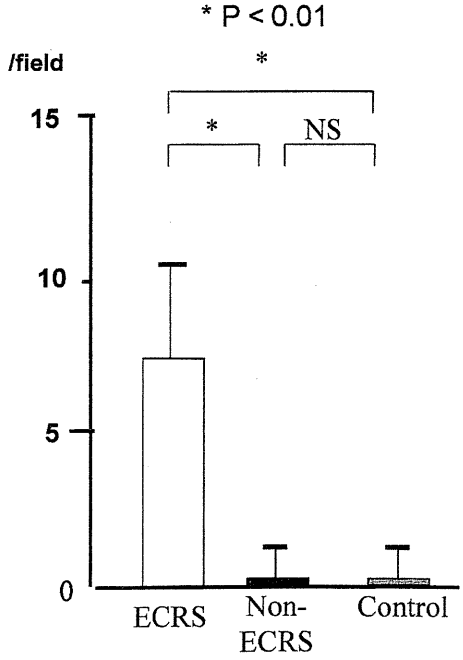

Fig. 8. The number of CD68 positive cells with the expression of HO-1 in epithelial layer. The number of CD68 positive cells with the expression of HO-1 in CRS with eosinophilic infiltration is significantly greater than CRS without eosinophilic infiltration and controls. *$p < 0.01$; NS: not significant.

4. Discussion

Vento et al. [6] revealed that eosinophilic infiltration into both serum and tissue were found most often in patients with acetylsalicylic acid intolerance and that such patients had the most active nasal polyposis as judged by the degree of sinus involvement, number of re-operations, and use of medication. Tissue eosinophilic infiltration might thus explain the higher recurrence and greater need for surgical treatment due to the increased activity of nasal polyposis.

Several investigators have reported fundamental and clinical studies of CRS with eosinophilic infiltration. The bronchus or alveoli of the lung as well as the sinonasal fossa comprise the respiratory airway with ciliated epithelia. Nonaka et al. [11] described that eosinophilic infiltration of tissue is a hallmark of nasal polyposis and asthma. They showed structural abnormalities such as fibrosis, thickening of the basement membrane, and detachment of the epithelium with areas of epithelial metaplasia. In fact, we previously reported that epithelial damage and basement membrane thickness in nasal polyps with CRS were correlated with the numbers of eosinophils [12]. It was also proposed that a central feature of CRS is the presence of an inflammatory process in which eosinophils are the most prevalent cell type. Eosinophils have some cytotoxic mediators as eosinophilic peroxidase, which can cause severe damage to the epithelia [13]. Moreover, the main source of metalloproteinases in asthmatic airways is believed to be eosinophils [14].

For migration through the basement membrane, eosinophils together with the other inflammatory cells secrete metalloproteinases to degrade collagens, a main component of the basement membrane [15]. Wenzel et al. [16] demonstrated that the endobronchial tissues in severe asthma showed increases in macrophages, which can produce oxidants [17]. Ijima et al. [18] described that oxidants aggravated nasal allergy-like symptoms by inducing nasal hyperresponsiveness and infiltrating eosinophils. The above reports support our view that infiltrating eosinophils and macrophages in the mucosa of CRS with eosinophilic infiltration would cause detachment or denudation of epithelial layers, as in severe asthma.

Otterbein et al. [19] demonstrated the enhanced expression of HO-1 in macrophages from sputum of patients with asthma. Kitada et al. [20] found that HO-1 expression localized in alveolar macrophages was increased in allergic airway inflammation. Horvath et al. [21] suggested that the induction of HO-1 could be a cytoprotective function against oxidative stress in lung disorders. We found that the number of macrophages expressing HO-1 was significantly greater in CRS with eosinophilic infiltration than in CRS without eosinophilic infiltration. We consider that macrophages and epithelial cells produce HO-1 to protect themselves against oxidants generated from macrophages, which are prominent in the tissues of CRS with eosinophilic infiltration. Furthermore, the expression of HO-1 in the epithelia of CRS with eosinophilic infiltration was significantly suppressed as compared with CRS without eosinophilic infiltration. Conversely, the HO-1 positive rate of epithelial cells in CRS without eosinophilic infiltration was significantly greater than that in CRS with eosinophilic infiltration. Xia et al. [22] revealed that HO-1 could attenuate eosinophilic infiltration in bronchial alveolar lavage fluid from asthmatic mice. Kim et al. [23] assessed PG102 (reagent with anti allergic effects) in a murine asthma model. In PG102-treated mice showing improvement of the asthmatic symptoms, high level expression of HO-1 was observed in alveolar inflammatory cells. Pae et al. [24] described that up-regulation of the HO-1 pathway had a significant protective effect against airway inflammation, mucus hyper-secretion and hyper-responsiveness in a model of allergic asthma. Our findings and those of other reports suggest

two theories, as follows. First, attacking oxidants from eosinophils and macrophages may deplete HO-1 in the epithelia of CRS with eosinophilic infiltration. Second, cytotoxic factors secreted from eosinophils and macrophages in the epithelial layers may increase and overcome the cytoprotection derived from epithelial cells and other inflammatory cells in CRS with eosinophilic infiltration. Thus, the epithelial damage characteristically observed in both asthma and CRS with eosinophilic infiltration is thought to be brought about by the interaction between cytotoxic enzymes or cytokines and cytoprotective factors such as HO-1. In the future, we will examine whether infiltrating eosinophils in CRS with eosinophilic infiltration can promote the production of HO-1 in macrophages or not.

5. Conclusion

The present study revealed that the expression of HO-1 in epithelial layers of CRS without eosinophilic infiltration was significantly greater than that in CRS with eosinophilic infiltration. On the other hand, the number of HO-1 positive macrophages was significantly enhanced in CRS with eosinophilic infiltration as compared with CRS without eosinophilic infiltration. Epithelial detachment or denudation was observed in CRS with eosinophilic infiltration, but not in CRS without eosinophilic infiltration. The above findings suggest that both a reduction of HO-1 expression in epithelial cells and increased infiltration of macrophages positive for HO-1 are related to the epithelial damage of CRS with eosinophilic infiltration.

References

[1] Ponka P. Cell biology of heme. Am J Med Sci 1999;318:241–56.
[2] Choi AMK, Alam L. Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. Am J Respir Cell Mol Biol 1996;15:9–19.
[3] Horvath I, Loukides S, Wodehouse T, Kharitonov SA, Cole PJ, Baranes PJ. Increased levels of exhaled carbon monoxide in bronchiectasis: a new maker of oxidative stress. Thorax 1998;53:867–70.
[4] Suzuki H, Ikeda K, Honma R, Gotoh S, Oshima T, Furukawa M, et al. Prognostic factors of chronic rhinosinusitis under long-term low-dose macrolide therapy. ORL 2000;62:121–7.
[5] Suzuki H, Ikeda K. Mode of action of long-term low-dose macrolide therapy for chronic sinusitis in the light of neutrophil recruitment. Curr Drug Targets Inflamm Allergy 2002;1:117–26.
[6] Vento SI, Ertama LO, Hytonen EA, Wolff CHJ, Malmberg HO. Nasal polyposis clinical course during 20 years. Ann Allergy Asthma Immunol 2000;85: 209–14.
[7] Katten M, Gergen PJ, Eggleston P, Visness CM, Mitchell HE. Health effects of indoor nitrogen dioxide and passive smoking on urban asthmatic children. Allery Clin Immunol 2007;120:618–24.
[8] Marcinkiewicz J, Ciszek M, Bobek M, Strus M, Heczko PB, Kurnyta M, et al. Differential inflammatory mediator response in vitro from murine macrophages to lactobacilli and pathogenic intestinal bacteria. Int J Exp Pathol 2007;88:155–64.
[9] Elhini A, Abdelwanhab S, Ikeda K. Heme oxygenase (HO)-1 is upregulated in the nasal mucosa with allergic rhinitis. Laryngoscope 2006;119:446–50.
[10] Fokkens W, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps. Rhinology 2007;45:1–139.
[11] Nonaka M, Pawankar R, Saji F, Yagi T. Distinct expression of RANTES and GM-CSF by lipopolysaccharide in human nasal fibroblasts but not in other airway fibroblasts. Int Arch Allergy Immunol 1999;119:314–21.
[12] Saitoh T, Kusunoki T, Yao T, Kawano K, Kojima Y, Miyashita K, et al. Relationship between epithelial damage or basement membrane thickness and eosinophilic infiltration in nasal polyps with chronic rhinosinusitis. Rhinology 2009;47:275–9.
[13] Locksley RM, Wilson CB, Klebanoff SJ. Role for endogenous and acquired peroxidase in the toxoplasamacidal activity of murine and human mononuclear phagocytes. J Clin Invest 1982;69:1099–111.
[14] Togaya E, Tamaoki J. Mechanisms of airway remodeling in asthma. Allergol Int 2007;56:331–40.
[15] Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. Am J Respir Cell Mol Biol 1997;17:519–28.
[16] Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 1999;160:1001–8.
[17] Vendrow AE, Hakim ZS, Madamanchi NR, Rojas M, Madamanchi C, Runge MS. Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. Aterioscler Thromb Vasc Biol 2007; 27:2714–21.
[18] Iijima MK, Kobayashi T, Kamada H, Shimojo N. Exposure to ozone aggravates nasal allergy-like symptoms in guinea pigs. Toxicol Lett 2001;123:77–85.
[19] Otterbein L, Sylvester SL, Choi AM. Hemoglobin provides protection against lethal endotoxemia in rats: the role of heme oxygenase-1. Am J Respir Cell Mol Biol 1995;13:595–601.
[20] Kitada O, Kodama T, Kuribayashi K, Ihaku M, Fujita T, Matsuyama T, et al. Heme oxygenases-1 (HO-1) protein induction in a mouse model of asthma. Clin Exp Allergy 2001;31:1470–7.
[21] Horvath I, Donnelly LE, Kiss A, Paredi P, Kharitonov SA, Barnes PJ. Raised levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: a new marker of oxidative stress. Thorax 1998;53:668–72.
[22] Xia ZW, Xu LQ, Zhong WW, Wei JJ, Li NL, Shao J, et al. Heme oxygenase-1 attenuates ovalbumin-induced airway inflammation by up-regulation of foxp3 T-regulatory cells, interleukin-10, and membrane-bound transforming growth factor-1. Am J Pathol 2007;171:1904–14.
[23] Kim D, Kim SH, Park EJ, Kang CY, Cho SH, Kim S. Anti-allergic effects of PG102, a water-soluble extract prepared from *Actinidia arguta*, in a murine ovalbumin-induced asthma model. Clin Exp Allergy 2009;39:280–9.
[24] Pae HO, Lee YC, Chung HT. Heme oxygenase-1 and carbon monoxide: emerging therapeutic targets in inflammation and allergy. Recent Pat Inflamm Allergy Drug Discov 2008;2:159–65.

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Clinical Efficacy of Anti-IgE Therapy for Eosinophilic Otitis Media

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Objective: Eosinophilic otitis media (EOM) is an intractable otitis media characterized by a highly viscous effusion containing eosinophils, and high levels of immunoglobulin (Ig) E are detected in the middle ear effusion (MEE). We carried out a pilot study to determine whether anti-IgE therapy is efficacious in the treatment of EOM.
Study Design: Prospective study.
Setting: Tertiary referral center.
Patients and Methods: Eight patients with EOM received the anti-IgE agent omalizumab for at least 3 months, in addition to ordinary treatments for EOM. They were evaluated by a questionnaire for ear and respiratory symptoms, clinical scores, surrogate markers in the blood, and hearing acuity before and after the anti-IgE therapy. Nine EOM patients without anti-IgE therapy were included as controls.
Results: The ear symptom scores and clinical scores gradually decreased during the therapy. In particular, 5 patients who were treated for more than 1 year showed improvement of their clinical scores with resolution of the MEE. The total serum IgE level was significantly elevated after 3 months of therapy ($p < 0.01$). Deterioration of the bone conduction hearing levels was more frequently found in the control group than in the omalizumab group.
Conclusion: This pilot study provides new evidence establishing that long-term anti-IgE therapy improved the clinical ear symptoms of EOM and bone conduction hearing levels were mostly preserved. Therefore, long-term anti-IgE therapy can be effective for EOM to inhibit eosinophilic inflammation in the middle ear. **Key Words:** Anti-IgE therapy—Bronchial asthma—Eosinophilic otitis media—Immunoglobulin E—Omalizumab.

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Eosinophilic otitis media (EOM) is an intractable otitis media characterized by the presence of a highly viscous yellow effusion containing eosinophils. In 1993, Tomioka et al. (1) first reported 3 cases of patients with intractable otitis media associated with bronchial asthma as a new middle ear disease entity. EOM is resistant to conventional treatments for otitis media with effusion or chronic otitis media, such as insertion of a tympanostomy tube, administration of antimicrobial agents, or tympanoplasty. Not only is EOM an intractable and persistent disease, it also presents a high risk for development of severe hearing loss (deafness in some cases) (1–3). Immunohistologic studies have shown active inflammation with the production of various cytokines and chemokines that induce eosinophil migration in the middle ear mucosa (4–6). In addition, many IgE-immunopositive cells are found in the middle ear mucosa (4). In the middle ear effusion (MEE), the IgE levels are significantly higher in EOM patients than in control patients with common otitis media with effusion (7). The presence of high levels of IgE may exacerbate eosinophilic inflammation in the middle ear. Omalizumab, a recombinant humanized monoclonal anti-IgE antibody, is the first anti-IgE agent with clinical benefits in the treatment of moderate-to-severe bronchial asthma. Therefore, we carried out a pilot study to determine whether anti-IgE therapy is also efficacious in the treatment of EOM. This is the first report of a prospective study on the efficacy of anti-IgE therapy for EOM.

PATIENTS AND METHODS

Patients

Eight patients with EOM who received anti-IgE therapy with omalizumab were included in this study. The patients were 6 female and 2 male subjects aged 33 to 69 years (mean ± standard deviation [SD], 54.4 ± 11.7 yr) at the time of administration of omalizumab (omalizumab group). As controls, 9 patients with EOM without anti-IgE therapy were included in the study. These patients were

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The authors disclose no conflict of interest.

TABLE 1. *Baseline characteristics of the patients*

	Omalizumab group	Control group	*p* value
No. patients	8	9	
Sex (female : male)	6:2	6:3	NS
Age (yr, mean ± SD)	54.4 ± 11.7	56.9 ± 16.7	NS
Associated diseases			
Aspirin intolerance	4	3	NS
Chronic rhinosinusitis	8	7	NS
Nasal polyposis	5	6	NS
Bacterial infection in MEE	3	4	NS
Baseline mean scores (range)			
Ear symptom score	11.6 (7–21)	11.6 (3–23)	NS
ACT score	18.5 (13–24)	19.0 (12–25)	NS
Clinical score	7.8 (3–11)	6.7 (5–12)	NS
Treatments			
Inhaled corticosteroids	8	9	NS
Intranasal corticosteroids	7	8	NS
Intratympanic TA	8	9	NS
Systemic corticosteroids	7	8	NS
Eosinophil counts in PB (/μl)	537 ± 421	477 ± 156	NS
Total serum IgE level (IU/ml)	267 ± 203	295 ± 361	NS
ECP (μg/L)	29.8 ± 29.1	16.5 ± 5.5	NS

ACT indicates asthma control test; IgE, immunoglobulin E; ECP, eosinophilic cationic protein; MEE, middle ear effusion; NS, not significant; PB, peripheral blood; SD, standard deviation; TA, triamcinolone acetonide.

6 female and 3 male subjects aged 30 to 80 years (mean ± SD, 56.9 ± 16.7 yr; control group). All the patients had association with bronchial asthma and were diagnosed as having EOM on the basis of previously reported diagnostic criteria as follows (8). The major criterion was otitis media with effusion or chronic otitis media with eosinophil-dominant effusion. The minor criteria were as follows: 1) highly viscous MEE, 2) resistance to conventional treatment for otitis media, 3) association with bronchial asthma, and 4) association with nasal polyposis. Definitive cases were defined as positive for the major criterion plus 2 or more of the minor criteria. Regarding eosinophil detection in the MEE, formalin-fixed, paraffin-embedded MEE sections were evaluated to obtain information about eosinophil activation and degranulation in the effusion. The baseline characteristics of the EOM patients in both groups are shown in Table 1.

Treatments

Omalizumab was given to the 8 patients with EOM in the omalizumab group. The dosage of omalizumab was individually determined based on the pretreatment level of total serum IgE and the body weight, according to dosing tables that approximately reflected the following formula: 0.016 mg/kg per IU/ml of IgE per 4 weeks. The drug was administered by subcutaneous injections every 2 or 4 weeks. All the patients in both groups continued their previous treatments for bronchial asthma and EOM during the study. They were all treated with inhaled corticosteroids with or without long-acting beta 2-adrenergic agonists for bronchial asthma. They had also experienced frequent asthma exacerbations and continuously or frequently taken oral corticosteroids, in addition to inhaled therapy. For EOM, intratympanic instillation of triamcinolone acetonide (9) was 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. The baseline treatments for bronchial asthma and EOM in both groups are also summarized in Table 1. The baseline characteristics of the patients in both groups were almost the same. Written informed consent was obtained from all patients before enrollment in the study. This study was approved by the Ethics Committee of Jichi Medical University Saitama Medical Center (study number: RIN 10–11).

Measurements of Surrogate Markers in Blood

We monitored the eosinophil counts in peripheral blood and total serum IgE and eosinophilic cationic protein (ECP) levels before and after the therapy because they are reliable markers of eosinophilic inflammation. The serum concentrations of total IgE and ECP were measured using fluorescence enzyme immunoassays.

Evaluation of Hearing

The air and bone conduction hearing levels of the patients with EOM were assessed by pure tone audiometry. 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.

Evaluation of Clinical Efficacy

At every visit, all the patients in the omalizumab group were evaluated using a questionnaire consisting of 8 questions about ear symptoms (Table 2) and the asthma control test (ACT) consisting of 5 questions about respiratory symptoms (http://www.asthma.com/resources/asthma-control-test.html). The patients in the control group were also evaluated using the questionnaire and ACT at the time of enrollment in the study, at 3 months and at 1 year. The efficacy was also evaluated using the 5 items with scores of 0 to 2 points as the clinical scores of the patients in both groups at the time of enrollment in the study and after 3 months, 6 months, and 1 year. The following 3 items were evaluated separately for both ears: quantity of MEE or otorrhea, condition of the middle ear mucosa, and frequency of intratympanic injection of triamcinolone acetonide. The frequencies of administration of systemic corticosteroids and antibiotics to the patients were also evaluated. The definitions of the scores for each item are shown in Table 3.

Statistical Analysis

Statistical analyses were carried out using Student's *t* test, the Wilcoxon matched-pairs signed-ranks test and the χ^2 test. Values of $p < 0.05$ were considered significant. Values of $p < 0.10$ were considered to show a tendency toward significance because they may have become significant if more patients had been included in this study.

RESULTS

Anti-IgE Therapy

In the 8 patients in the omalizumab group, 3 patients stopped receiving omalizumab at 3 months, mostly for

TABLE 2. *Questionnaire for ear symptoms*

Have you had the following symptoms in the last 2 weeks?
Scores: 0, none; 1, rare; 2, sometimes; 3, often; 4, usually; 5, worst.
1 Echo in the ear
2 Tinnitus
3 Otorrhea
4 Dizziness
5 Breathing sound in the ear
6 Autophony
7 Aural fullness
8 Otalgia

TABLE 3. *Definitions of the scores for the 5 items for clinical efficacy*

Quantity of MEE/otorrhea	
Without eardrum perforation	
Score 0	No MEE
Score 1	MEE with partial intratympanic aeration
Score 2	Mesotympanum totally filled with MEE
With eardrum perforation	
Score 0	No otorrhea
Score 1	Otorrhea limited in the mesotympanum
Score 2	Otorrhea comes out to the external auditory canal
Condition of the middle ear mucosa	
Score 0	Nearly normal or slightly edematous
Score 1	Edematous or thickened
Score 2	Highly thickened or granulated to an extent beyond the position of a normal eardrum
Frequency of intratympanic administration of corticosteroid	
Score 0	None
Score 1	Once in the previous 3 months
Score 2	Two or more times in the previous 3 months
Frequency of systemic administration of corticosteroids	
Score 0	None
Score 1	7 days or less in the previous 3 months
Score 2	More than 7 days in the previous 3 months
Frequency of systemic administration of antibiotics	
Score 0	None
Score 1	7 days or less in the previous 3 months
Score 2	More than 7 days in the previous 3 months

economical reasons, and the remaining 5 patients continued the therapy for more than 1 year. No adverse events were seen during the administration of omalizumab in any patients.

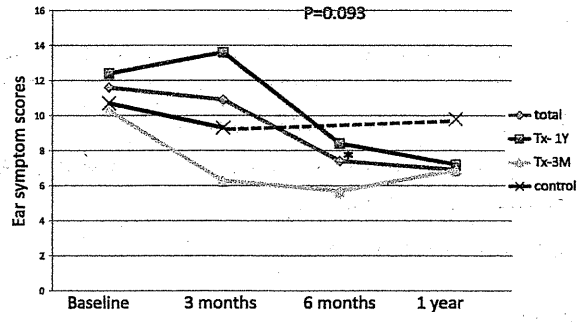

FIG. 1. Changes in the ear symptom scores evaluated by a questionnaire in the patients treated with omalizumab. Total: 8 patients treated with omalizumab; Tx-1Y: 5 patients treated with omalizumab for more than 1 year; Tx-3M: 3 patients treated with omalizumab for 3 months; control: 9 patients without administration of omalizumab.

Ear Symptom Score Evaluation by the Questionnaire

The ear symptom score was calculated as the sum of the scores of the 8 questions shown in Table 2. The changes in the average ear symptom scores of the patients in the omalizumab group are shown in Figure 1. The symptom scores gradually decreased during the therapy. The mean score at 6 months was 7.4, which showed a tendency to be reduced compared with the baseline level of 11.6 in the 8 patients ($p = 0.093$). However, the scores in the 3 patients who stopped administration of omalizumab at 3 months had slightly increased again at 1 year. In the control group, the significant changes of the scores from the baseline were not observed during the study.

ACT Score

In the ACT, a score of 25 indicates perfectly controlled bronchial asthma, and scores of lower than 20 indicate poorly controlled asthma. The changes in the mean ACT scores of the patients in the omalizumab group are shown in Figure 2. The mean scores of the 8 patients increased from 18.5 at baseline to 23.4 at 3 months ($p = 0.092$). Individually, 4 patients in the omalizumab group with initial ACT scores of lower than 20 showed improved scores of higher than 20 after 1 year of the therapy. Finally, all the patients except one showed scores of higher than 20 at 1 year, indicating well-controlled asthma. In contrast, the mean ACT scores of the patients in the control group were 19.0 at baseline and 21.2 at 3 months, with no statistical significance.

Clinical Score

The changes in the clinical scores, which were evaluated by 3 items for the bilateral ear conditions and 2 items for the frequencies of systemic administration of corticosteroids and antibiotics, were analyzed and are shown in Figure 3.

The total scores were significantly reduced after administration of omalizumab for 3 months and 1 year compared with the baseline score (Fig. 3F). In particular, the mean scores for the quantity of MEE tended to be reduced after the treatment (Fig. 3A). Five patients who were

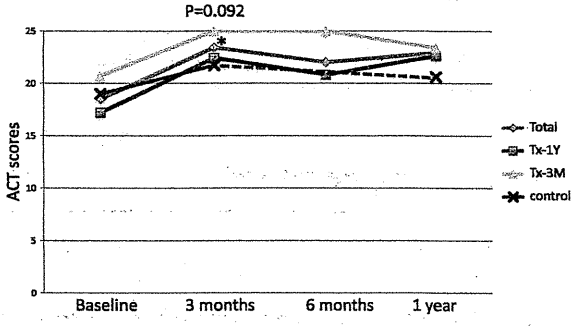

FIG. 2. Changes in the ACT scores of the patients treated with omalizumab. Total: 8 patients treated with omalizumab; Tx-1Y: 5 patients treated with omalizumab for more than 1 year; Tx-3M: 3 patients treated with omalizumab for 3 months; control: 9 patients without administration of omalizumab.

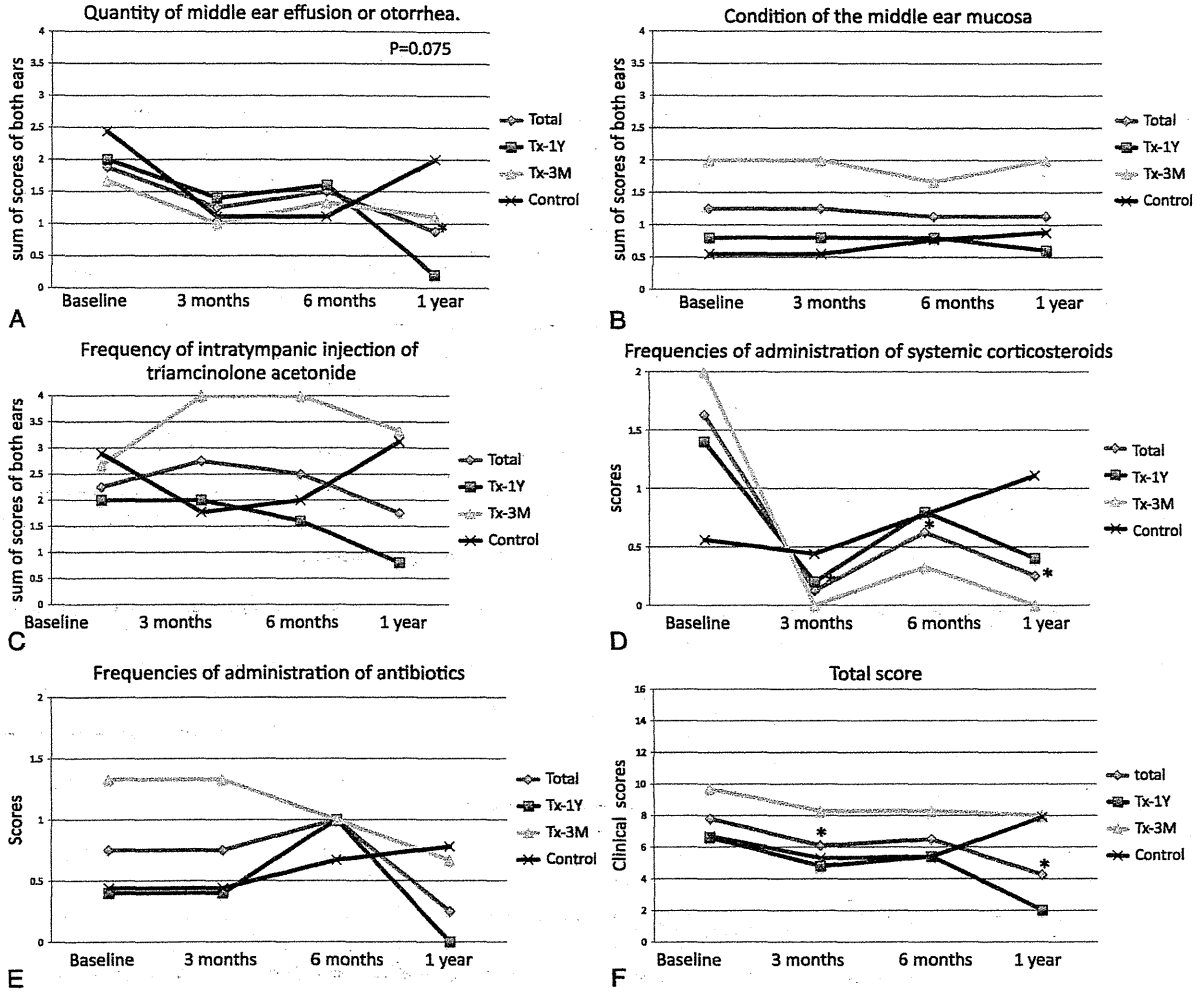

FIG. 3. Changes in the clinical scores of the patients with EOM. Quantity of MEE or otorrhea (*A*), condition of the middle ear mucosa (*B*), frequency of intratympanic injection of triamcinolone acetonide (*C*), frequency of administration of systemic corticosteroids (*D*), frequency of administration of antibiotics (*E*), and total scores (*F*). The scores of the first 3 items represent the sums of the scores for both ears. Total: 8 patients treated with omalizumab; Tx-1Y: 5 patients treated with omalizumab for more than 1 year; Tx-3M: 3 patients treated with omalizumab for 3 months; control: 9 patients without administration of omalizumab.

treated more than 1 year showed resolution of the MEE or otorrhea for a long period and seldom needed intratympanic instillation of corticosteroids after 1 year. However, the condition of the middle ear mucosa remained almost the same (Fig. 3B). The frequency of administration of systemic corticosteroids was significantly reduced after the treatment (Fig. 3D). In the control patients, the mean clinical scores at each point showed no significant changes from the baseline level for the total score and the scores of the 5 items.

Eosinophil Counts in Peripheral Blood and Concentrations of Total Serum IgE and ECP

These findings are shown in Figure 4. The eosinophil counts in the peripheral blood tended to reduce after treatment in the omalizumab group, particularly at 3 months ($p = 0.096$), whereas the mean eosinophil counts showed no significant change in the control group. The 5 patients who continued administration of omalizumab also showed a reduction in the eosinophil counts at 3 months ($p = 0.096$). The concentration of total serum IgE was significantly elevated after 3 months of omalizumab administration ($p < 0.01$) and then gradually decreased in patients treated not only for 3 months but also for 1 year. In contrast, the control patients did not show elevation of the total serum IgE level, that is, 260 IU/ml (SD, 349) at baseline and 253 IU/ml (SD, 320) at 3 months, with no significant difference. The serum ECP concentration in the omalizumab group gradually decreased from the baseline level. Although the patients treated for 3 months showed a reduction in the serum ECP level at 3 months, the level was elevated again at 1 year.

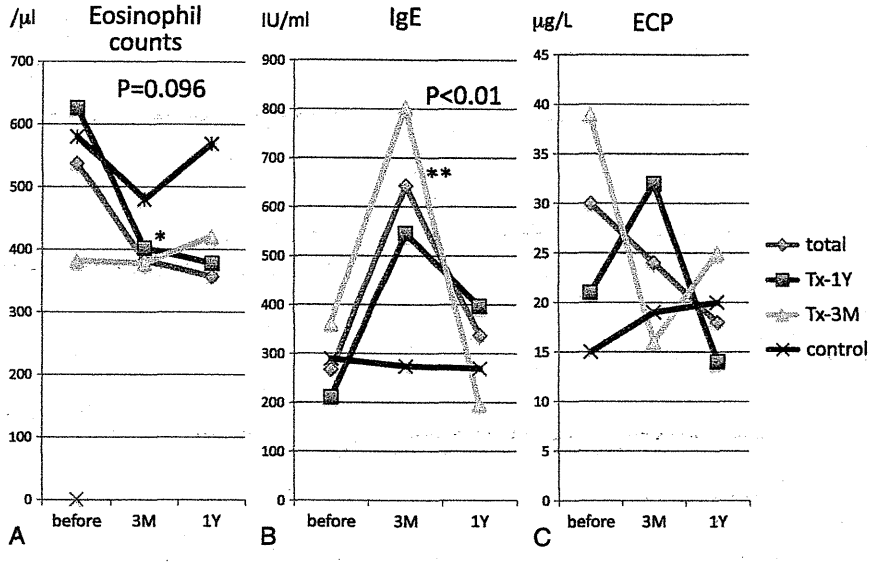

FIG. 4. Changes in the peripheral blood eosinophil counts, serum IgE, and serum ECP of the patients treated with omalizumab. Total: 8 patients treated with omalizumab; Tx-1Y: 5 patients treated with omalizumab for more than 1 year; Tx-3M: 3 patients treated with omalizumab for 3 months; control: 9 patients without administration of omalizumab.

Bone Conduction Hearing Level

These findings are shown in Table 4. The EOM patients in the omalizumab group as well as the control group did not show significant deterioration of the BCHLs at the mean speech range and 4 kHz at 1 year after enrollment in the study. However, in the omalizumab group, 1 patient showed bilateral deterioration of the BCHL beyond 15 dB at the mean speech range or 4 kHz. On the other hand, in the control group, the BCHL worsened in 7 ears of 4 patients at the mean speech range or 4 kHz at 1 year ($p = 0.081$).

Efficacy of Anti-IgE Therapy

The sums of the ear symptom score and the clinical score at baseline and 1 year after enrollment were evaluated in each patient. In the omalizumab group, 5 patients were regarded as responders to the therapy because their total scores were reduced to less than two-thirds from the baseline at 1 year. Two of the 3 nonresponders were patients who stopped the therapy at 3 months.

DISCUSSION

Anti-IgE therapy was first included in Step 5 of the Global Initiative for Asthma Guideline (10) in 2006 as an add-on treatment to inhaled and eventually oral corticosteroids and other controller medications. A recent meta-analysis of 8 trials (n = 3429 participants) showed the efficacy and safety of omalizumab in adults, adolescents, and children with moderate-to-severe asthma (11). Anti-IgE therapy has been applied not only for bronchial asthma but also for chronic idiopathic urticaria and seasonal allergic rhinitis, and the efficacy of the therapy has been reported (12–14). Patients with bronchial asthma are often associated with chronic rhinosinusitis with nasal polyposis. This condition is called eosinophilic chronic rhinosinusitis, which is sometimes refractory and tends to recur even after endoscopic sinus surgery. Recently, the effectiveness of anti-IgE therapy for chronic rhinosinusitis-associated bronchial asthma has been reported (15). Although that article was only a case report, surprisingly, the patient also showed mastoiditis on magnetic resonance imaging that resolved completely after 4 months of anti-IgE therapy together with amelioration of the symptoms of bronchial asthma and chronic rhinosinusitis. The mastoiditis in the patient would undoubtedly have been EOM. EOM is now recognized as an intractable otitis media and is a fairly common middle ear disease not only in Japan but also worldwide. The condition is very intractable, and the patients have persistent MEE/otorrhea and hearing loss, resulting in a worsening quality of life. Currently, the most effective treatment for EOM is administration of topical corticosteroids (9). Patients need periodic intratympanic instillation of corticosteroids and sometimes show exacerbation and require administration of oral corticosteroids. Thus far, complete and long-term resolution of MEE/otorrhea has been quite rare. This is the first study to evaluate the effectiveness of anti-IgE therapy with omalizumab for EOM associated with bronchial asthma.

TABLE 4. *Bone conduction hearing levels of the patients with eosinophilic otitis media (dB)*

	Omalizumab group		Control group	
	Speech range	4 kHz	Speech range	4 kHz
Baseline	15.6 ± 11.0	28.1 ± 21.7	21.2 ± 15.4	26.9 ± 19.9
1 year	19.4 ± 13.4	32.5 ± 21.9	28.4 ± 17.8	35.0 ± 24.3
p value	NS	NS	NS	NS

Speech range: mean hearing level at 0.5, 1, and 2 kHz.

In this study, we found evidence that anti-IgE therapy can be effective if patients are administered omalizumab for more than 1 year, although the number of patients was limited. The objective scores determined by the questionnaire for ear symptoms and clinical scores clearly showed improvement of EOM. Although omalizumab is indicated for moderate-to-severe allergic asthma, Menzella et al. (16) reported a significant effect of omalizumab in patients with severe nonallergic asthma. The EOM patients enrolled in this study had association with both allergic and nonallergic asthma, and both diseases responded relatively well. Our data showed dramatic reductions in the clinical scores of the patients treated with omalizumab at 1 year from baseline, with reduced quantities of MEE/otorrhea and decreased frequencies of systemic administration of corticosteroids, particularly in the 5 patients treated for more than 1 year.

Eosinophilia is a typical characteristic of asthma-related inflammatory diseases, including EOM. A meta-analysis of 5 randomized, double-blind, placebo-controlled studies showed that significant reductions in the eosinophil counts from baseline were observed in patients with allergic asthma treated with omalizumab compared with those administered placebo (17). In our study, the eosinophil counts tended to decrease after 3 months of the anti-IgE therapy, whereas the concentration of ECP, a cytotoxic protein derived from eosinophils, failed to show significant decreases after the therapy. The precise mechanism that induces the reduction in eosinophils remains to be determined. However, Noga et al. (18) reported increased eosinophil apoptosis through reduced production of granulocyte-macrophage–colony-stimulating factor, an important factor for eosinophil growth and survival, after administration of omalizumab.

The total serum IgE level was elevated from baseline at 3 months and then gradually decreased in this study. By binding to free IgE, omalizumab prevents interactions between IgE and its high-affinity FcεRI receptors on mast cells and basophils, such that IgE cross-linking and cell activation cannot occur. In our study, we measured the concentration of total serum IgE, which is the sum of circulating free IgE and omalizumab-bound IgE. Owing to the binding of the monoclonal antibody to IgE, the total serum IgE level usually tends to increase progressively during omalizumab treatment. A recent study showed that early elevation of 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 (19).

One of the striking clinical characteristics of EOM was the high incidence of gradual or sudden deterioration of hearing. In our previous study, 8 (5.8%) of 138 patients became deaf unilaterally or bilaterally after the onset of EOM (8). High-tone loss was more frequently encountered and more severe in EOM patients than in age-matched patients with chronic otitis media (20). Our previous study also showed that the concentration of IgE in MEE was significantly and positively correlated with the BCHLs at 2 and 4 kHz and that a high concentration of IgE in the MEE as well as a high serum IgE level were significant risk factors for BCHL deterioration in EOM patients (7). Therefore, anti-IgE therapy can prevent BCHL deterioration. In this study, although the mean BCHLs at the speech range and 4 kHz did not significantly change at 1 year from baseline in the omalizumab group as well as in the control group, the number of patients showing BCHL deterioration of more than 15 dB from baseline tended to be higher in the control group than in the omalizumab group. These findings indicate that the administration of omalizumab could prevent inner ear damage to control the eosinophilic inflammation.

In conclusion, from a limited number of patients with EOM, we provide the first report that long-term anti-IgE therapy could improve their ear symptoms and decrease the systemic administration of corticosteroids. Randomized controlled trials are necessary to formally confirm these findings and to determine the factors for responders to the therapy.

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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*. New York, NY: Kugler Publications, 1993:183–6.
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, 1997:851–3.
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. 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;31:1135–43.
5. 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;123:383–7.
6. Iino Y, Kakizaki K, Katano H, Saigusa H, Kanegasaki S. Eosinophil chemoattractant in middle ear of patients with eosinophilic otitis media. *Clin Exp Allergy* 2005;35:1370–6.
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; 31:100–4.
8. 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;38:456–61.
9. 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.
10. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. Available from: http://www.ginathma.org. Accessed May 3, 2012.
11. Rodrigo GJ, Neffen H, Castro-Rodriguez JA. Efficacy and safety of subcutaneous omalizumab vs placebo as add-on therapy to corticosteroids for children and adults with asthma: a systematic review. *Chest* 2011;139:28–35.
12. Saini S, Rosen KE, Hsieh H-J, et al. A randomized, placebo-controlled, dose-ranging study of single-dose omalizumab in patients

with H1-antihistamine-refractory chronic idiopathic urticaria. *J Allergy Clin Immunol* 2011;128:567–73.
13. Plewako H, Arvidsson M, Petruson K, et al. The effect of omalizumab on nasal allergic inflammation. *J Allergy Clin Immunol* 2002;110:68–71.
14. Vignola AM, Humbert M, Bousquer J, et al. Efficacy and tolerability of anti-immunoglobulin E therapy with omalizumab in patients with concomitant allergic asthma and persistent allergic rhinitis SOLAR. *Allergy* 2004;7:709–17.
15. Grundmann SA, Hemfort PB, Luger TA, Brehler R. Anti-IgE (omalizumab): a new therapeutic approach for chronic rhinosinusitis. *J Allergy Clin Immunol* 2008;121:257–8.
16. Menzella F, Piro R, facciolongo N, Castgnetti C, Simonazzi A, Zucchi L. Long-term benefits of omalizumab in a patients with severe non-allergic asthma. *Allergy Asthma Clin Immunol* 2011;7:9.
17. Massanari M, Holgate ST, Busse WW, et al. Effect of omalizumab on peripheral blood eosinophilia in allergic asthma. *Respir Med* 2010;104:188–96.
18. Noga O, Hanf G, Brachmann I, et al. Effect of omalizumab treatment on peripheral eosinopil and T-lymphocyte function in patients with allergic asthma. *J Allergy Clin Immunol* 2006;117:1493–9.
19. Dal Negro RW, Guerriero M, Micheletto C, Ognella S, Visconti M. Changes in total IgE plasma concentration measured at the third month during anti-IgE treatment predict future exacerbation rates in difficult-to-treat atopic asthma: a pilot study. *J Asthma* 2011;48: 437–41.
20. 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.

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◆特集・耳鼻咽喉科における抗ウイルス薬・ステロイドの効果的処方

好酸球性中耳炎の診断と治療
—特に局所ステロイドの使い方—

吉田尚弘[*1]　飯野ゆき子[*2]

好酸球性中耳炎は，好酸球の浸潤を特徴とする粘性が極めて高いニカワ状の耳漏あるいは中耳貯留液を認める難治性中耳炎である．感音難聴の進行，最終的に聾に至ることがあり臨床上大きな問題となる．

好酸球性中耳炎には，① 滲出性中耳炎型，② 慢性中耳炎型に分けられる．治療の基本は，本疾患を念頭に置いた診断とステロイドの中耳腔内への局所投与，全身投与，感染に対する抗菌薬投与である．局所ステロイドの効果発現には中耳腔粘膜への浸潤が必要である．トリアムシノロンアセトニド(ケナコルト®)，デキサメタゾン(デカドロン®)，リンデロンなどが用いられるが，トリアムシノロンアセトニドが比較的効果の持続期間が長い．本稿では好酸球性中耳炎の診断，治療，特に局所ステロイドの使用方法について述べる．

好酸球性中耳炎(eosinophilic otitis media)，ステロイド(steroid)，トリアムシノロンアセトニド(triamcinolone acetonide)，好酸球(eosinophil)，中耳炎(otitis media)

はじめに

好酸球性中耳炎は，好酸球の浸潤を特徴とする粘性が極めて高いニカワ状の耳漏あるいは中耳貯留液を認める難治性中耳炎である．

感音難聴の進行，最終的に聾に至ることがあり臨床上大きな問題となる．好酸球の関与する難治性上気道炎として，好酸球性中耳炎のほか，気管支喘息，好酸球性副鼻腔炎，Churg-Strauss 症候群などがある．それらの疾患概念を図1に示した．好酸球性中耳炎，好酸球性副鼻腔炎ともに好酸球浸潤を伴う難治性上気道炎に位置づけられるが，その病態，原因，関連については不明な点が多い．

好酸球性中耳炎の提唱

1993 年富岡(松谷)らによって気管支喘息に合併した難治性中耳炎症例が報告され[1]，その後，好酸球を含む粘稠な貯留液を有する難治性滲出性中耳炎が報告された[2]．1997 年富岡らは7症例をまとめて報告し，アトピー性素因が証明されないにもかかわらず中耳貯留液に著明な好酸球浸潤を認める病態から，好酸球性中耳炎“eosinophilic otitis media”いう名称を提唱した[3]．一方，欧米では 1950 年頃から Derlacki，Shambaugh らにより，アレルギーが関与する慢性中耳炎“allergic otitis media”が報告，提唱された[4)~6)]．中耳貯留液には好酸球の著明な浸潤を認める点では，本邦の好酸球性中耳炎とは組織学的特徴は類似する．しかし，治療にはステロイド内服までは必ずしも必要とせず，抗アレルギー薬の治療に反応する．本邦の好酸球性中耳炎は抗アレルギー薬のみでの治療は困難である．この“allergic otitis media”の臨床像は治療抵抗性，投与薬剤の点から本邦の好酸球性中耳炎と比べて，比較的軽症である印象をうける．“allergic otitis media”は，本邦の好酸球性中耳炎“eosinophilic otitis media”とは，アレル

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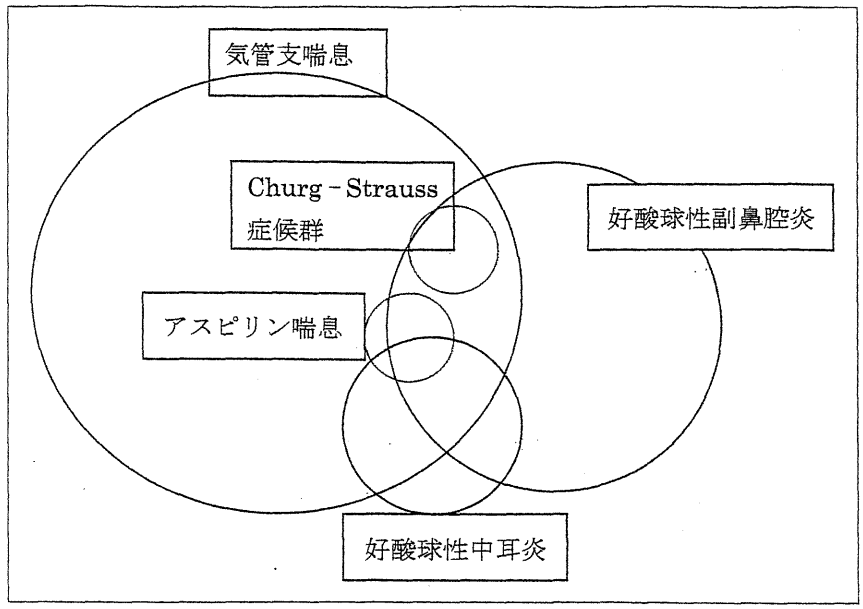

図 1.
好酸球の関与する難治性上気道炎の疾患概念

ギーが関与しているという点では共通であるがより広い『好酸球，アレルギーの関与する中耳炎』という疾患概念の範疇にあると考えてよいかもしれない．

好酸球性中耳炎の診断

比較的新しい疾患概念である好酸球性中耳炎の治療を論じていく上では，その診断基準を示していく必要がある．

鈴木らによって2003年好酸球性中耳炎の全国疫学調査が行われた[7]．その際の診断基準は，疑い例；成人の気管支喘息患者にみられるニカワ状の耳漏を特徴とする，薬物療法，鼓膜チューブ留置術，手術に抵抗性の難治性中耳炎．確実例；疑い例でかつ耳漏または中耳粘膜，肉芽に著しい好酸球浸潤が認められた症例であった．この疫学調査によれば，好酸球性中耳炎確実例341例，疑い例446例，二次調査でその臨床像が明らかとなった症例は190例であった．初診年齢は50歳代，60歳代，40歳代が多く女性が61％と男性に比べて有意に高かった．

中耳貯留液に好酸球浸潤をきたす難治性中耳炎の個々の症例を詳細に検討していくと，気管支喘息を合併することが多いが，必ずしも合併しない症例もあることがわかった．このことは，好酸球性中耳炎は，好酸球浸潤の伴う中耳炎であって，その疾患カテゴリーにはいくつかの要因の病態が混在していることを示している．

表 1. 好酸球性中耳炎診断基準

大項目
好酸球優位な中耳貯留液が存在する滲出性中耳炎／慢性中耳炎
小項目
①ニカワ状の貯留液
②中耳炎に対する従来の治療に抵抗
③気管支喘息の合併
④鼻茸の合併
確実例：大項目＋小項目2つ以上
除外例：Churg-Strauss 症候群，好酸球増多症候群

そこで，本邦から提唱された診断基準を表1に示す[8]．

大項目として好酸球優位の中耳貯留液を有する滲出性中耳炎／慢性中耳炎，小項目として ①ニカワ状の貯留液，②一般的治療に抵抗性，③気管支喘息の合併，④鼻茸の合併があり，大項目をみたし且つ2つ以上の小項目を満たせば確実例とした．しかし，この条件を満たしても，Churg-Strauss症候群，好酸球増多症候群は除外する．

この診断基準では，中耳貯留液の好酸球浸潤は診断には必須である．したがって，鼓膜穿孔のない耳漏のない一見滲出性中耳炎と鑑別される症例では鼓膜切開し，中耳貯留液の好酸球浸潤の証明が必要となる．

今後，診断基準，疾患概念が国内外で統一されていくものと考えている．

好酸球性中耳炎の臨床的特徴

疫学調査によれば，初診年齢は50歳代，60歳

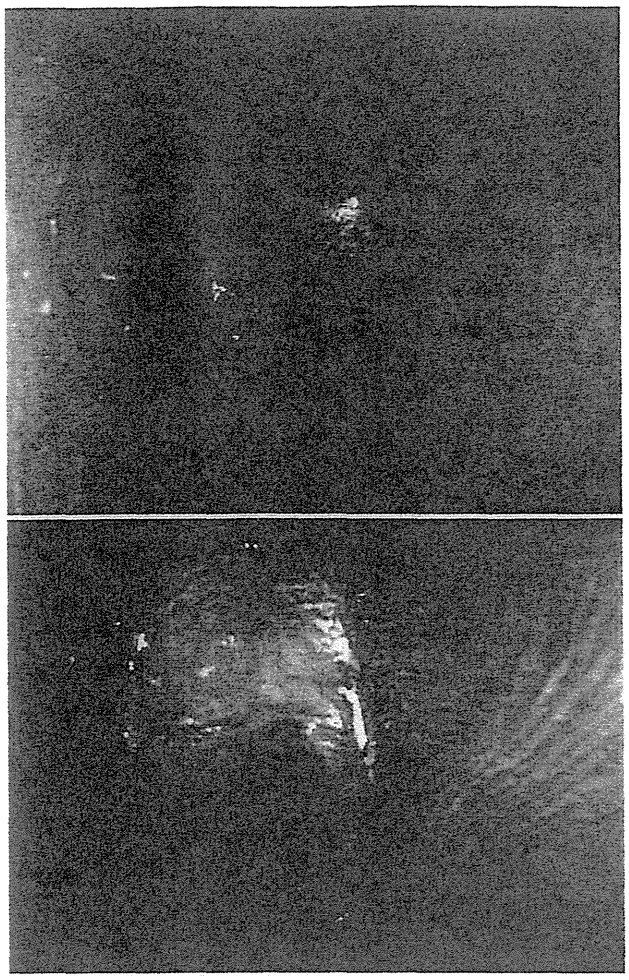

図 2. 好酸球性中耳炎の鼓膜所見
a：滲出性中耳炎型の左鼓膜所見
b：慢性穿孔性中耳炎型：肉芽の生じた左鼓膜所見

代，40歳代が多く女性が61%と男性に比べて有意に高かった[7]．自験例47例のうち29例(62%)が女性，50歳代が最多と同等であった．両側を罹患することが多く80%を超える．自験例でも47例中40例(87%)は両側性であった．

通常成人発症型の気管支喘息との合併を認める．自験例では98%，アスピリン喘息(AIA)との合併は23%，副鼻腔炎との合併は85%であった．鈴木らの疫学調査でも74%の副鼻腔炎の合併を認めている．

側頭骨CTでみられる乳突洞の発育は一般に良い例が多く，幼少時の中耳炎の既往がないことが多い．

鼓膜所見から大きく①鼓膜穿孔のないもの(滲出性中耳炎型)，②鼓膜穿孔のあるもの(慢性穿孔性中耳炎型)に分けられる．

①滲出性中耳炎型：多くの場合鼓膜はやや黄色を呈する．鼓膜切開を行うと粘稠な中耳液貯留を認め，時として太めの吸引管での吸引が必要となる．難治性滲出性中耳炎で特に気管支喘息合併例ではまず好酸球性中耳炎を疑って検査する必要がある．鼓膜換気チューブを留置しても貯留液により内腔が閉塞，また，自然排出されてしまうことがあり有用ではないため基本的には挿入しない．

②慢性穿孔性中耳炎型：鼓膜穿孔は自然に穿孔が形成されることがあり，また鼓膜切開，鼓膜穿刺により形成した穿孔が閉鎖しないこともある．穿孔の大きさは様々であるが，中耳粘膜はほとんど肥厚を認めないものから，浮腫上，瘢痕上に肥厚したもの，外耳道にまで高度の肉芽増生を認めることがある．感染を生ずることもあり注意を要する．

初期は伝音難聴であるが，経過中に混合性難聴，時には聾となることがあり臨床上大きな問題となる．高音域より，聴覚閾値の上昇を呈し[9]．罹病期間と8000 Hzの骨導閾値上昇が相関することが報告されている[10]．疫学調査では47%に骨導閾値の上昇を認め，そのうち6%が聾となった．感音難聴をきたす原因は不明であるが，内耳窓から，炎症性産物や細菌由来のtoxinが内耳へ波及し生じてくると考えられている．

好酸球性中耳炎の病態

好酸球性中耳炎発症の要因の一つに，この耳管機能，耳管粘膜，鼻腔，気管支喘息の状態が関わっている．気道粘膜がTh2優位の状態にあるとき耳管経由で好酸球遊走，活性化に関わる因子が進入することを契機として中耳粘膜で好酸球炎症が引き起こされていると考えられる．実際，耳管機能検査を行うと，耳管開放時間の延長を有している症例が多い[11]．中耳粘膜の杯細胞からムチン増生が盛んとなりニカワ状の中耳貯留液が生じる．この中耳貯留液には好酸球浸潤に関連するIL-5，eotaxin，好酸球由来の細胞障害性蛋白であるeosinophil cationic protein(ECP)が血清よりも高値を示す[12)~14)]．この好酸球遊走のトリガーとなる因