



Contents lists available at ScienceDirect

Allergy International

journal homepage: <http://www.elsevier.com/locate/alit>

Original article

Eosinophil-derived neurotoxin, elastase, and cytokine profile in effusion from eosinophilic otitis media

Hirota Uchimizu^{a,*}, Yoshinori Matsuwaki^a, Masahiko Kato^b, Nobuyosi Otori^a, Hiromi Kojima^a^a Department of Otorhinolaryngology, The Jikei University School of Medicine, Tokyo, Japan^b Department of Pediatrics, Tokai University School of Medicine, Kanagawa, Japan

ARTICLE INFO

Article history:

Received 11 January 2015

Received in revised form

28 March 2015

Accepted 30 March 2015

Available online xxx

Keywords:

Cytokine profile

Elastase

Eosinophil-derived neurotoxin

Eosinophilic otitis media

Secretory otitis media

ABBREVIATIONS:

BAL, bronchoalveolar lavage; bFGF, basic fibroblastic growth factor; CRS, chronic rhinosinusitis; ELISA, enzyme-linked immunosorbent assay; EDN, eosinophil-derived neurotoxin; EOM, eosinophilic otitis media; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN- γ , interferon γ ; IL, interleukin; IP-10, interferon-inducible protein-10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF-BB, platelet-derived growth factor-BB; RANTES, regulated upon activation, normal T expressed and secreted; SOM, secretory otitis media; Th, helper T cell; TNF- α , tumor necrosis factor α ; VEGF, vascular endothelial growth factor

ABSTRACT

Background: Eosinophilic otitis media (EOM) is an intractable disease characterized by a remarkably viscous effusion and accumulation of numerous eosinophils in both the middle ear effusion and the mucosa. The key factors in EOM pathogenesis remain unclear. The purpose of this study is to identify the important factors involved in EOM pathogenesis.

Methods: Middle ear effusion samples were collected from 12 patients with EOM and 9 patients with secretory otitis media (SOM), as controls. Multiple cytokines in the effusion were measured using a Bio-Plex™ Human Cytokine 27-Plex panel. Eosinophil-derived neurotoxin (EDN) and elastase were measured by ELISA. The concentrations of EDN, elastase, and each cytokine were compared between the EOM and SOM groups. Furthermore, in the EOM group, each cytokine was examined for correlation with EDN and elastase.

Results: EDN and elastase concentrations were significantly higher in the EOM group than in the SOM group ($p < 0.05$). IL-5, IL-1 β , MIP-1 α , G-CSF, IL-1 α , IL-4, IFN- γ , MIP-1 β , IL-10, TNF- α , VEGF, and IL-2 concentration was significantly higher in the EOM group than in the SOM group ($p < 0.05$). Significant positive correlations were found between EDN and IL-1 α , IL-2, IL-5, IL-9, IL-13, eotaxin, MIP-1 α , PDGF-BB, and RANTES in the EOM group ($p < 0.05$).

Conclusions: Our study showed that IL-5, IL-2, MIP-1 α , and IL-1 α are the important factors involved in EOM pathogenesis. Furthermore, not only eosinophil, but also neutrophil are involved in middle ear inflammation of EOM.

Copyright © 2015, Japanese Society of Allergy. Production and hosting by Elsevier B.V. All rights reserved.

Introduction

Eosinophilic otitis media (EOM) is an intractable disease characterized by a remarkably viscous effusion and accumulation of numerous eosinophils in both the middle ear effusion and the mucosa. EOM is associated with adult-onset bronchial asthma, whether atopic or non-atopic, and chronic rhinosinusitis (CRS) with nasal polyps showing accumulation of numerous

* Corresponding author. Department of Otorhinolaryngology, The Jikei University School of Medicine, 3-25-8 Nishishimbashi, Minato-ku, Tokyo 105-8461, Japan.

E-mail address: uchimizu@jikei.ac.jp (H. Uchimizu).

Peer review under responsibility of Japanese Society of Allergy.

eosinophils. Other EOM clinical characteristics are (1) bilateral otitis media, (2) resistance to conservative treatments other than steroids, and (3) no association with type I allergy. In EOM, the chronic, primarily eosinophilic, inflammation is thought to occur in the middle ear mucosa, which is an extension of the respiratory tract mucosa.¹ Furthermore, at the hearing level, EOM may cause the deterioration of the bone-conduction hearing level^{2,3} and, occasionally, leads to deafness that requires cochlear implantation.⁴

CRS with nasal polyps is characterized by a type 2 helper T cell (Th2) cytokine profile. It is thought that interleukin 5 (IL-5) and eotaxin are the most important factors causing eosinophil accumulation in nasal polyps.^{5–8} Therefore, EOM is thought to present a cytokine profile similar to that of CRS with nasal polyps. Some studies have been designed to determine the relationship between EOM and eosinophil-active cytokines.^{9,10} These studies reported the presence of IL-5 and eotaxin in the middle ear effusion. However, only few cytokines were examined. Thus, which cytokines and/or chemokines are the most important factors in EOM pathogenesis remains unclear.

In this study, we found that IL-5, IL-2, macrophage inflammatory protein-1 α (MIP-1 α), and IL-1 receptor antagonist (IL-1ra) were important factors involved in EOM pathogenesis. These cytokines may have potential as therapeutic targets for EOM treatment such as anti-IL-5 antibody therapy.

Methods

Patients

All patients were treated at the Department of Otorhinolaryngology of The Jikei University Hospital. Middle ear effusion samples were collected from patients who had given written informed consent for participation in the study. The study was conducted in accordance with the ethical standards of Jikei University. EOM diagnosis was based upon the following criteria proposed by Nagamine et al.⁹: (1) presence of yellow and extremely viscous middle ear effusion containing predominantly eosinophils and (2) precedence and association with adult bronchial asthma (atopic or non-atopic). In this study, middle ear effusion samples were obtained from 12 patients with EOM who met the above diagnostic criteria. In addition, like EOM, adult secretory otitis media (SOM) is a chronic inflammatory disease that shows accumulation of an effusion in the middle ear without any accompanying bacterial infection or acute inflammation. In the early stage of EOM onset, the findings can be very similar to SOM. For this reason, SOM is a disease that is very difficult to distinguish from EOM. Accordingly, middle ear effusion samples were obtained from nine patients with adult SOM without bronchial asthma or chronic rhinosinusitis as controls.

Patients who were diagnosed as having asthma by an internal medicine specialist in the respiratory organs and were administered drugs for asthma were designated as having asthma. A diagnosis of aspirin-intolerant asthma was made from an episode of the onset of asthma attack after using non-steroidal anti-inflammatory drugs. A diagnosis of CRS with nasal polyps was made on the basis of an endoscopic examination and a CT scan. In addition, allergen-specific IgE antibodies for five inhalant allergens (house dust, mites, cedar pollen, *Alternaria alternate*, and *Aspergillus fumigatus*) were measured by using CAP-RAST and allergen-specific IgE values of 0.7U_A/mL or higher were considered positive. Atopy was defined as a positive response to at least one of these five allergens.

Collection of middle ear effusion samples

According to the tympanic membrane findings, EOM is classified into two types. The chronic otitis media type with tympanic membrane perforation and SOM type without tympanic membrane perforation. Middle ear effusion samples were collected from patients with EOM through the tympanic membrane perforation using a Jun-Tym-Tap[®] middle ear fluid aspirator/collector (Medtronic Xomed, Jacksonville, FL, USA) or after myringotomy via the external ear canal. Middle ear effusion samples were collected from patients with SOM after myringotomy. After dilution with an equal volume of saline and vortexing, effusion samples were stored below –20 °C until used.

Measurement of cytokine, EDN, and elastase

After thawing at room temperature, effusion samples were diluted 1:10 with 0.5% human serum albumin in phosphate-buffered saline. After thorough vortexing, the effusion samples were centrifuged at 2500 rpm for 5 min at 4 °C. The supernatants were assayed for multiple cytokines using the Bio-Plex[™] Human Cytokine 27-Plex panel (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The panel includes the following cytokines: IL-1 β , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic fibroblastic growth factor (bFGF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon γ (IFN- γ), interferon-inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), MIP-1 α , MIP-1 β , platelet-derived growth factor-BB (PDGF-BB), regulated upon activation, normal T expressed and secreted (RANTES), tumor necrosis factor α (TNF- α), and vascular endothelial growth factor (VEGF). As respective indicators of eosinophil and neutrophil activation, eosinophil-derived neurotoxin (EDN) and neutrophil elastase were measured with an EDN enzyme-linked immunosorbent assay (ELISA; MBL, Nagoya, Japan) and human PMN elastase ELISA (Bender MedSystems, Vienna, Austria), respectively, according to the manufacturers' recommendations. The concentrations of EDN, elastase, and each cytokine in the effusion were compared between the EOM and SOM groups. Furthermore, each cytokine was examined for possible correlation with EDN and elastase in the EOM group.

Statistical analysis

All statistical analyses were performed using the statistical software SPSS version 16 (IBM SPSS Japan Inc., Tokyo, Japan). The EOM and SOM groups were compared by Mann–Whitney's *U*-test. Correlations between variables were analyzed using Spearman's correlation coefficient by rank test. Differences with a *p*-value of less than 0.05 were considered statistically significant.

Results

Patient background characteristics in the EOM and SOM groups

Table 1 presents the data on the patient background characteristics in both the EOM and SOM groups. The groups showed no significant differences with regard to the number of patients, age, or gender. The peripheral eosinophil count and percentage were significantly higher in the EOM group than in the SOM group (*p* < 0.01), but the total serum IgE level showed no significant difference between the groups. In the EOM group, nine patients presented the chronic otitis media type with tympanic

Table 1

Patient background characteristics in the EOM and SOM groups.

	EOM	SOM
Patients (n)	12	9
Age (years)	51.1 ± 11.0	54.7 ± 12.3
Male/Female	7/5	5/4
Peripheral eosinophils		
Count, / μ L	1051.8 ± 1302.6 [†]	183.5 ± 150.6
Percentage	13.4 ± 13.1 [†]	2.6 ± 1.4
Total serum IgE, IU/mL	813.4 ± 1266.7	265.3 ± 108.1
Atopy	8/12	3/9
Bronchial asthma	12/12	0/9
Aspirin-intolerant asthma	1/12	0/9
CRS with nasal polyps	12/12	0/9
Tympanic membrane perforation	9/12	0/9
Steroid therapy		
Topical eardrops	6/12	0/9
Systemic	0/0	0/9

Data are expressed as mean ± standard deviation.

EOM, eosinophilic otitis media; SOM, secretory otitis media; CRS, chronic rhinosinusitis.

[†] Significant difference between the EOM and SOM groups ($p < 0.01$).

membrane perforation. In the EOM group, six patients had been treated with topical steroid eardrops, but nobody had been treated with systemic steroid therapy. On the other hand, nobody had been treated with either topical or systemic steroid therapy in the SOM group.

Comparison of end and elastase concentrations between the EOM and SOM groups

EDN and elastase concentrations in the effusion were compared between the EOM and SOM groups (Fig. 1). EDN concentration was significantly higher in the EOM group than in the SOM group ($p < 0.001$). Furthermore, elastase concentration was significantly higher in the EOM group than in the SOM group ($p < 0.05$).

Comparison of cytokine concentrations between the EOM and SOM groups

Each cytokine concentration in the effusion was compared between the EOM and SOM groups (Fig. 2). The concentration of IL-5, IL-1 β , MIP-1 α , G-CSF, IL-1 α , IL-4, IFN- γ , MIP-1 β , IL-10, TNF- α , VEGF, and IL-2 (in descending order of p -value) was significantly higher in the EOM group than in the SOM group ($p < 0.05$). However, there

was no statistical significant difference in eotaxin or RANTES concentrations between the EOM and SOM groups. On the other hand, IP-10 concentration was significantly higher in the SOM group than in the EOM group ($p < 0.01$).

Histopathological finding of middle ear effusion from patient with EOM

The histopathological finding of middle ear effusion from patient with EOM is shown in Fig. 3. Numerous inflammatory cells infiltrated in the middle ear effusion. Not only numerous eosinophils (black arrows), but also numerous neutrophils (white arrows) were observed.

Correlations between EDN and each cytokine in the EOM group

Significant positive correlations were found between EDN and IL-1 α , IL-2, IL-5, IL-9, IL-13, eotaxin, MIP-1 α , PDGF-BB, and RANTES in the EOM group ($p < 0.05$) (Table 2).

Correlations between elastase and each cytokine in the EOM group

Significant positive correlations were found between elastase and IL-6 and PDGF-BB in the EOM group ($p < 0.05$), while a negative correlation was found with TNF- α ($p < 0.05$) (Table 2).

Discussion

Previous EOM studies examined only a few cytokines such as IL-5, eotaxin, and RANTES.^{9,10} This is the first report to show the simultaneous assay of multiple cytokines and comparison with not only an indicator of eosinophil activation such as EDN, but also an indicator of neutrophil activation such as neutrophil elastase. In this study, IL-5, IL-2, MIP-1 α , and IL-1 α concentration was significantly different between the EOM and SOM groups and correlated with EDN in the EOM group (Fig. 4). This result suggests that these four cytokines are specifically involved in EOM pathogenesis.

CRS with nasal polyps is characterized by intense eosinophilic inflammation and high IL-5 levels in the nasal tissue. Therefore, IL-5 is an important factor for eosinophil accumulation and activation in CRS with nasal polyps.^{5–7} Furthermore, IL-5 is a key factor not only in CRS with nasal polyps, but also in several eosinophil-associated disorders. Clinical trials of anti-IL-5 antibody therapy have been performed for these disorders.^{11–13} Similar to these eosinophil-associated disorders, our results also suggest that IL-5 is an important factor for eosinophil accumulation in the middle ear in EOM. This result is in agreement with that of a previous study,¹⁰ which reported that the concentration of eosinophil cationic protein was positively correlated with that of IL-5 and that IL-5 may play an important role in the recruitment of eosinophils in the middle ear. On the other hand, it is interesting that not only IL-5, which is a Th2 cytokine, but also IL-2, which is a Th1 cytokine, is also involved in EOM pathogenesis. In addition, IFN- γ concentration in the EOM group was also significantly higher than in the SOM group. Thus, the cytokine profile in the effusion from patients with EOM included both Th2 and Th1 cytokines. It has generally been thought that eosinophilic inflammation is associated with Th2 immune responses and that nasal polyps are characterized by a Th2 cytokine profile. However, several studies reported that the cytokine pattern in nasal polyps is not Th2 cytokine-predominant, but a mixed type, including upregulation of both Th2 and Th1.^{14–16} Similarly, our results suggest that both Th2 and Th1 cytokines are also important factors in EOM pathogenesis.

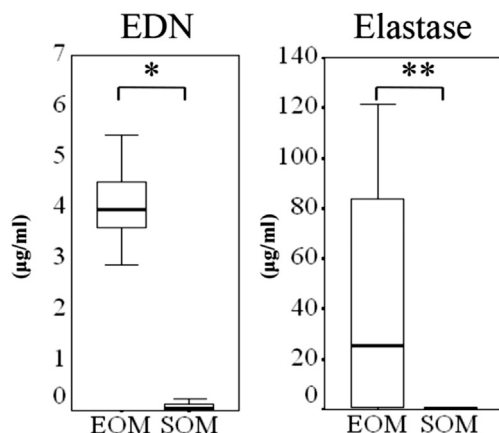


Fig. 1. Comparison of EDN and elastase concentrations between the EOM and SOM groups. * $p < 0.001$, ** $p < 0.05$. EDN, eosinophil-derived neurotoxin.

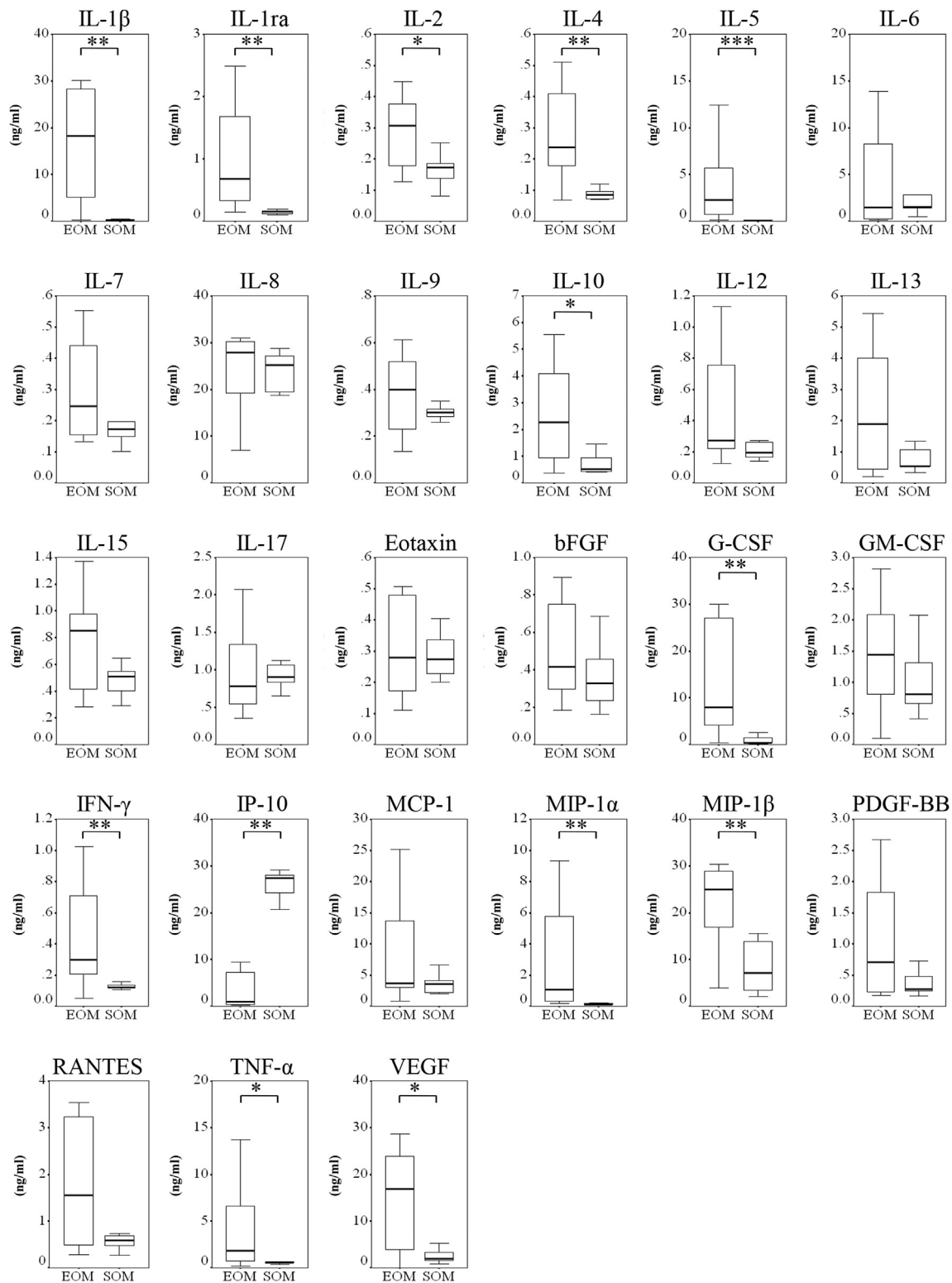


Fig. 2. Comparison of cytokines concentrations between the EOM and SOM groups * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. IL, interleukin; bFGF, basic fibroblastic growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN- γ , interferon γ ; IP-10, interferon-inducible protein-10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF-BB, platelet-derived growth factor-BB; RANTES, regulated upon activation, normal T expressed and secreted; TNF- α , tumor necrosis factor α ; VEGF, vascular endothelial growth factor.

MIP-1 α is a chemokine that binds to C–C chemokine receptor-1, which is expressed on neutrophils, eosinophils, monocytes, T lymphocytes, and basophils, with high affinity. It is confirmed that MIP-1 α was increased in the bronchoalveolar lavage (BAL) fluid

from bronchial asthma after an allergen challenge.¹⁷ MIP-1 α also induces eosinophil migration and, like RANTES, MIP-1 α is an important mediator of the inflammatory process in which eosinophils predominate.¹⁸ Furthermore, MIP-1 α and RANTES are major

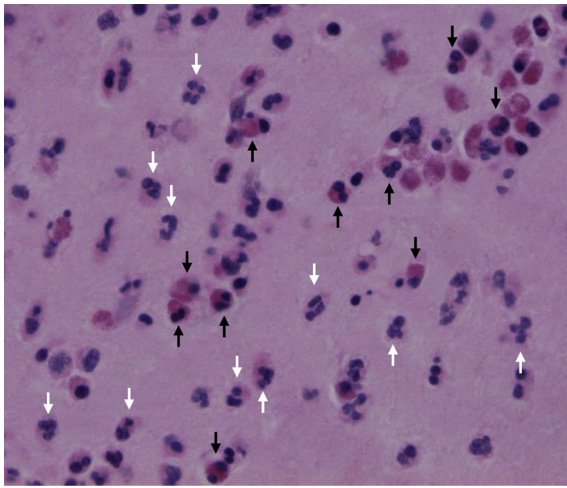


Fig. 3. Histopathological finding of the middle ear effusion from patient with EOM (Hematoxylin and Eosin staining, $\times 400$). Numerous eosinophils (black arrows) and neutrophils (white arrows) are observed.

chemotactic proteins involved in eosinophil accumulation during allergy airway responses.¹⁹ Our results indicate that MIP-1 α is a more important factor involved in eosinophil accumulation in EOM than RANTES.

IL-1ra is an anti-inflammatory cytokine that blocks the action of IL-1 α and IL-1 β functional ligands by competitive inhibition at the IL-1 receptor level.²⁰ Therefore, when IL-1ra is produced

excessively compared to IL-1, IL-1 activity is controlled and, as a result, the inflammatory reaction may be suppressed. Our results indicate that IL-1ra is one of the important factors involved in EOM pathogenesis. Compared to patients with adult respiratory distress syndrome, patients with acute eosinophilic pneumonia had high IL-1ra BAL fluid levels, which decreased after the resolution of the symptoms and completion of a course of corticosteroids.²¹ Furthermore, IL-1 induced eosinophil accumulation in the skin. This eosinophil accumulation was blocked by IL-1ra.²² In EOM, IL-1ra may play an important role in suppressing the eosinophilic inflammation in the middle ear.

In our study, both EDN and elastase concentrations were significantly higher in the EOM group than in the SOM group. These results indicate that not only eosinophils, but also neutrophils were recruited at the same time in EOM. Actually, both numerous eosinophils and neutrophils were observed in the middle ear effusion from patient with EOM, histopathologically. This finding indicates that both eosinophils and neutrophils are likely involved in the EOM middle ear inflammation. In the clinic, when EOM symptoms worsen such as an increase of the viscous effusion, we sometimes see a response not only to steroids, but also to antibiotic eardrops. This finding on the concentration of elastase supports our above-noted clinical experience. We surmise that an additional bacterial infection might be a factor aggravating the middle ear inflammation in EOM. However, the nine patients that had a tympanic membrane perforation among the 12 patients with EOM may be easily infected with bacteria via the external ear canal compared to the patients without tympanic membrane perforation. Therefore, further comparative studies will be needed in patients with EOM without tympanic membrane perforation.

Only the concentration of IP-10, which is a cytokine that is expressed in response to infection by various viruses,^{23,24} was significantly higher in the SOM group than in the EOM group. Recently, we have reported that serum IP-10 was elevated in acute exacerbation of childhood asthma infected by rhinovirus and respiratory syncytial virus.²⁵ Like asthma, pediatric otitis media is known to be associated with viral upper respiratory tract infection.²⁶ On the other hand, the pathogenesis of adult SOM is not yet well understood and relationship between adult SOM and viral infection has not been pointed out. Therefore, our finding suggests that viral upper respiratory tract infections may be involved in the pathogenesis of adult SOM.

In the year 2011, Iino et al. reported the new diagnostic criteria for EOM. So far, there were no established diagnostic criteria for EOM.²⁷ When this study started, these new diagnostic criteria were not established yet. Therefore, in this study, we used the criteria proposed by Nagamine et al.,⁹ which focus on typical patients with EOM. However, not all patients with EOM have bronchial asthma or eosinophilic rhinosinusitis. In the new diagnostic criteria, the presence of eosinophil-dominant effusion is the major criterion. On the other hand, highly viscous middle ear effusion, resistance to conventional treatments for otitis media, association with bronchial asthma, and association of nasal polyposis are minor criteria. A patient with a major item and, at least, two minor items is diagnosed with EOM. Even if these new diagnostic criteria were used, all patients recruited in our study would be diagnosed with EOM and we support these new diagnostic criteria for EOM.

In conclusion, our study showed that IL-5, IL-2, MIP-1 α , and IL-1ra are specifically involved in EOM pathogenesis of. These cytokines may have potential as therapeutic targets for EOM such as anti-IL-5 antibody therapy. Not only anti-IL-5 antibody, but IL-1ra has also been tested in clinical trials for several disorders.^{28–30} Therefore, these may also be effective for EOM treatment.

Table 2
Correlation between each cytokine and EDN or elastase in the EOM group.

	EDN		Elastase	
	r	p Value	r	p Value
IL-1 β	–0.124	0.717	–0.450	0.163
IL-1ra	0.725	0.008	0.087	0.789
IL-2	0.755	0.007	0.044	0.897
IL-4	0.185	0.584	–0.230	0.495
IL-5	0.654	0.021	0.085	0.792
IL-6	0.507	0.092	0.648	0.023
IL-7	0.401	0.196	0.269	0.398
IL-8	0.137	0.672	–0.030	0.926
IL-9	0.658	0.028	0.018	0.958
IL-10	–0.033	0.924	–0.097	0.777
IL-12	0.376	0.254	0.397	0.227
IL-13	0.769	0.006	0.163	0.632
IL-15	0.495	0.102	0.231	0.471
IL-17	0.544	0.068	0.371	0.236
Eotaxin	0.755	0.005	0.131	0.684
bFGF	0.278	0.381	0.137	0.670
G-CSF	0.149	0.661	0.146	0.668
GM-CSF	0.395	0.204	–0.248	0.437
IFN- γ	0.311	0.325	0.230	0.472
IP-10	0.508	0.110	0.560	0.073
MCP-1	0.026	0.939	–0.087	0.800
MIP-1 α	0.739	0.009	0.003	0.992
MIP-1 β	0.241	0.475	–0.247	0.463
PDGF-BB	0.733	0.007	0.661	0.019
RANTES	0.822	0.001	0.277	0.387
TNF- α	–0.382	0.220	–0.674	0.016
VEGF	–0.011	0.973	0.085	0.792

r, correlation coefficient; IL, interleukin; bFGF, basic fibroblastic growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN- γ , interferon γ ; IP-10, interferon-inducible protein-10; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF-BB, platelet-derived growth factor-BB; RANTES, regulated upon activation, normal T expressed and secreted; TNF- α , tumor necrosis factor α ; VEGF, vascular endothelial growth factor; EDN, eosinophil derived neurotoxin.

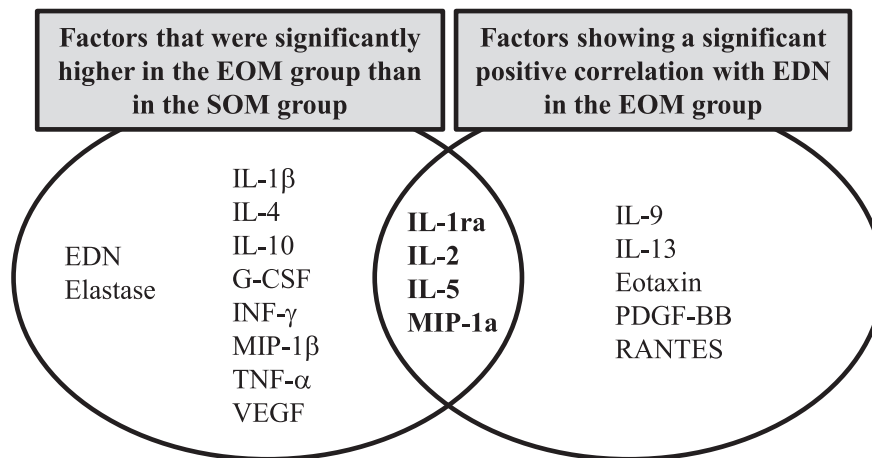


Fig. 4. Possible important factors involved in the pathogenesis of EOM. IL, interleukin; G-CSF, granulocyte-colony stimulating factor; INF-γ, interferon γ; IP-10, interferon-inducible protein-10; MIP, macrophage inflammatory protein; PDGF-BB, platelet-derived growth factor-BB; RANTES, regulated upon activation, normal T expressed and secreted; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor; EDN, eosinophil-derived neurotoxin.

However, most studies on EOM are still at the early stages and much further investigation is necessary. In the near future, EOM pathogenesis should be sufficiently clarified to allow the development of the therapeutic tools needed by physicians to prevent deafness due to EOM.

Acknowledgments

The authors thank Masakazu Yoshizumi, M.S., Gunma Prefectural Institute of Public Health and Environmental Sciences, for technical support in the assay.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

HU and YM designed the study and collected data. HU wrote the manuscript. YM performed the statistical analysis. All authors performed the interpretation of the results, read the manuscript, and approved the final manuscript.

References

- Matsutani S. Concept and diagnosis of eosinophilic otitis media. *Otol Jpn* 2004;**14**:108–11 [in Japanese].
- Iino Y, Usubuchi H, Komada K, Takizawa K, Kanazawa T, Ohta Y. Bone conductive hearing level in patients with eosinophilic otitis media associated with bronchial asthma. *Otol Neurotol* 2008;**29**:949–52.
- Iino Y, Usubuchi H, Kodama K, Takizawa K, Kanazawa T, Ohta Y. Eosinophilic inflammation in the middle ear induces deterioration of bone-conduction hearing level in patients with eosinophilic otitis media. *Otol Neurotol* 2009;**31**:100–4.
- Iwasaki S, Nagura M, Mizuta K. Cochlear implantation in a patient with eosinophilic otitis media. *Eur Arch Otorhinolaryngol* 2006;**263**:365–9.
- Bachert C, Gevaert P, Holtappels G, Cuvelier C, van Cauwenberge P. Nasal polyposis: from cytokines to growth. *Am J Rhinol* 2000;**14**:279–90.
- Riechelmann H, Deutshle T, Rozsasi A, Keck A, Polzehl D, Bärner H. Nasal biomarker profiles in acute and chronic rhinosinusitis. *Clin Exp Allergy* 2005;**35**:1186–91.
- van Zele T, Claeys S, Gevaert P, van Maele G, Holtappels G, van Cauwenberge P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy* 2006;**61**:1280–9.
- Lamblin C, Bolard F, Gosset P, Tsicopoulos A, Perez T, Darras J, et al. Bronchial interleukin-5 and eotaxin expression in nasal polyposis: relationship with (a) symptomatic bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 2001;**163**:1226–32.
- 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.
- Iino Y, Kakizaki K, Katano H, Saigusa H, Kanegasaki S. Eosinophil chemotactic factors in the middle ear of patients with eosinophilic otitis media. *Clin Exp Allergy* 2005;**35**:1370–6.
- Corren J. Anti-interleukin-5 antibody therapy in asthma and allergies. *Curr Opin Allergy Clin Immunol* 2011;**11**:565–70.
- Molfino NA. Targeting of eosinophils in asthma. *Expert Opin Biol Ther* 2012;**12**:807–9.
- Corren J. Inhibition of interleukin-5 for the treatment of eosinophilic diseases. *Discov Med* 2012;**13**:305–12.
- Bachert C, Gevaert P, Holtappels G, van Cauwenberge P. Mediators in nasal polyposis. *Curr Allergy Asthma Rep* 2002;**2**:481–7.
- Danielsen A, Tynning T, Brokstad KA, Olofsson J, Davidsson A. Interleukin 5, IL6, IL12, INF-γ, RANTES and fractalkine in human nasal polyps, turbinate mucosa and serum. *Eur Arch Otorhinolaryngol* 2006;**263**:282–9.
- Otto BA, Wenzel SE. The role of cytokines in chronic rhinosinusitis with nasal polyps. *Curr Opin Otolaryngol Head Neck Surg* 2008;**16**:270–4.
- Holgate ST, Bodey KS, Janecz A, Frew AJ, Kaplan AP, Teran LM. Release of RANTES, MIP-1α, and MCP-1 into asthmatic airway following endobronchial allergen challenge. *Am J Respir Crit Care Med* 1997;**156**:1377–83.
- Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA. RANTES and macrophage inflammatory protein 1 alpha induce the migration and activation of normal human eosinophil granulocyte. *J Exp Med* 1992;**176**:1489–95.
- Lukacs NW, Standiford TJ, Chensue SW, Kunkel RG, Strieter RM, Kunkel SL. C-C chemokine-induced eosinophil chemotaxis during allergic airway inflammation. *J Leukoc Biol* 1996;**60**:573–8.
- Opal SM, DePalo VA. Anti-inflammatory cytokines. *Chest* 2000;**117**:1162–72.
- Allen JM, Liao Z, Wewers MD, Altenberger EA, Moore SA, Allen ED. Detection of IL-5 and IL-13 receptor antagonist in bronchoalveolar lavage fluid in acute eosinophilic pneumonia. *J Allergy Clin Immunol* 1996;**97**:1366–74.
- Sanz MJ, Weg VB, Bolanowski MA, Nourshargh S. IL-1 is a potent inducer of eosinophil accumulation in rat skin. Inhibition of response by a platelet-activating factor antagonist and an anti-human IL-8 antibody. *J Immunol* 1995;**154**:1364–73.
- Liu MT, Chen BP, Oertel P, Buchmeier MJ, Armstrong D, Hamilton TA, et al. Cutting edge: the T cell chemoattractant IFN-inducible protein 10 is essential in host defense against viral-induced neurologic disease. *J Immunol* 2000;**165**:2327–30.
- Liu MT, Armstrong D, Hamilton TA, Lane TE. Expression of Mig (monokine induced by interferon-γ) is important in T lymphocyte recruitment and host defense following viral infection of the central nervous system. *J Immunol* 2001;**166**:1790–5.
- Kato M, Tsukagoshi H, Yoshizumi M, Saitoh M, Kozawa K, Yamada Y, et al. Different cytokine profile and eosinophil activation are involved in rhinovirus- and RS virus-induced acute exacerbation of childhood wheezing. *Pediatr Allergy Immunol* 2011;**22**:e87–94.
- Doyle WJ, Alper CM. Prevention of otitis media caused by viral upper respiratory tract infection: vaccines, antivirals, and other approaches. *Curr Allergy Asthma Rep* 2003;**3**:326–34.
- Iino Y, Tomioka-Matsutani S, Matsubara A, Nakagawa T, Nonaka N. Diagnostic criteria of eosinophilic otitis media, a newly recognized middle ear disease. *Auris Nasus Larynx* 2011;**38**:456–61.
- Cain BS, Meldrum DR, Harken AH, McIntyre Jr RC. The physiological basis for anticytokine clinical trials in the treatment of sepsis. *J Am Coll Surg* 1998;**186**:337–50.
- Smolen JS, Aletaha D, Koeller M, Weisman MH, Emery P. New therapies for treatment of rheumatoid arthritis. *Lancet* 2007;**370**:1861–74.
- Goldbach-Mansky R. Blocking interleukin-1 in rheumatic diseases. *Ann N Y Acad Sci* 2009;**1182**:111–23.