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IgG4 Expression in Patients with Eosinophilic Otitis Media

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Keywords
Eosinophilic otitis media · Otitis media · IgG4 · IgG4 staining · Hearing loss · Cochlear implant

Abstract
Objective: Eosinophilic otitis media (EOM) is an intractable middle ear disease recognized by an eosinophil enriched middle ear effusion and mucosa. Although precise pathogenesis of EOM remains unclear, it is characterized by type 2 inflammation. Since IgG4 is an IgG subclass induced by type 2 cytokines such as IL-4 and IL-13, we sought to characterize and compare local IgG4 expression in patients with and without EOM. ***Methods:*** Twelve patients with bilateral profound hearing loss, 9 of which underwent a cochlear implant surgery, were enrolled in this study (6 with EOM and 6 without EOM). The surgical specimens were harvested during surgery and were subjected to IgG4 immunostaining. ***Result:*** The middle ear mucosa showed the presence of a large number of IgG4-positive cells in patients with EOM, which was significantly higher than that in patients without EOM. ***Conclusion:*** Local IgG4 expression was observed in patients with EOM in comparison to those without EOM, suggesting that IgG4 contributes to EOM pathogenesis.

Introduction

Eosinophilic otitis media (EOM) is an intractable middle ear disease in which the ear effusion and mucosa contain abundant eosinophils [1]. EOM is characterized by type 2 inflammation recognized by eosinophil activation, mucin production, and IgE production. The type 2 inflammation is induced by the action of type 2 cytokines such as IL-4, IL-5, and IL-13 mainly produced by $CD4^+$ T helper type 2 (Th2) cells and type 2 innate lymphoid cells (ILC2s). In the upper airway including middle ear, IL-4, IL-5, and IL-13 were associated with IgE production, eosinophil activation, and tissue remodeling, respectively.

The deterioration of bone conductive hearing level (BCHL) is more frequently observed in patients with EOM than in those with chronic otitis media [2]. About 47% of EOM patients show deterioration in BCHL and 6% develop profound sensorineural hearing loss (SNHL) despite undergoing conservative therapy – intratympanic, topical steroid administration [3]. However, its pathogenesis has not been fully elucidated. It has been reported that cochlear implant (CI) surgery is safe and provides good speech recognition in patients with EOM and deafness [4].

Of the 4 human IgG subclasses, IgG4 is the least abundant in serum (approximately 5%) of total IgG [5]. Simi-

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Table 1. Diagnostic criteria of EOM

Major criterion
Otitis media with effusion or chronic otitis media with eosinophil-dominant effusion
Minor criteria
Highly viscous middle ear effusion
Resistance to conventional treatment for otitis media
Association with bronchial asthma
Association with nasal polyposis

A patient who shows otitis media with effusion or chronic otitis media with eosinophil-dominant effusion (major criterion) and with 2 or more among the highest 4 items (minor criteria) can be diagnosed as having eosionophilic otitis media. Churg-Strauss syndrome and hypereosinophilic syndrome were diagnoses by exclusion. EOM, eosinophilic otitis media.

lar to IgE, IgG4 is associated with type 2 inflammation since its production is induced by type 2 cytokines such as IL-4 and IL-13 [6]. For example, we have reported that the number of IgG4-positive plasma cells is significantly higher in patients with eosinophilic chronic rhinosinusitis (ECRS), one of the typical type 2 inflammatory diseases, than in those with conventional non-ECRS. The local IgG4 expression is associated with disease severity and treatment outcome in chronic rhinosinusitis, especially ECRS [7].

The pathogenesis of EOM overlaps with that of ECRS and these conditions coexist [8]. To our knowledge, the local expression of IgG4 in EOM has not yet been reported. In the present study, we determined local IgG4 expression in patients with EOM and compared it to those without EOM.

Materials and Methods

Patients

Six patients diagnosed with EOM according to the established criteria were included in this study [9] (Table 1). All the patients suffered bilateral profound SNHL due to EOM and 3 of them underwent a CI surgery through a round window approach at our institutions. The remaining 3 patients had worn bilateral hearing aids and had been taken the specimen through the perforated tympanic membrane. All the patients concomitantly suffered from chronic rhinosinusitis with nasal polyps and bronchial asthma and received inhalable corticosteroids. As control, 6 more patients who suffered bilateral profound SNHL due to progressive idiopathic SNHL with no inflammation in middle ear were included (non-EOM). They also received a CI through a round window approach. Tissue specimens from the mastoid antrum were collected during the surgery. This study was approved by the Institutional Review Board of International University of Health and Welfare, Mita hospital (No. 5-18-10). Informed written consent was obtained from all the patients for using the tissue specimens in this study.

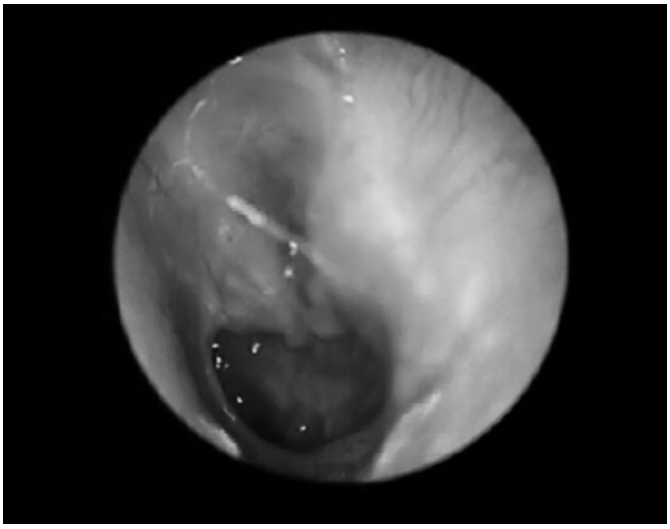

Fig. 1. Tympanic membrane of EOM: edematous mucosa with granulation tissue through perforation. EOM, eosinophilic otitis media.

Pathological Examination and Immunohistochemistry

The surgical specimens were fixed in 10% formaldehyde and embedded in paraffin. Serial sections (4 μm) were cut from each paraffin-embedded tissue block and stained with hematoxylin and eosin. IgG4 immunostaining was also performed. In brief, immunohistochemistry was performed on paraffin sections using an automated Bond-Max stainer (Leica Biosystems, Melbourne, Australia) with antihuman IgG4 mAb (1:400; HP6025, The Binding Site, Birmingham, UK) as the primary antibody. IgG4-positive cells were estimated for areas with the highest density of positive cells. Three different high-power fields (HPFs) in each section were counted by a pathology reviewer blinded to the patient characteristics, and the average number of positive cells per HPF was determined.

Statistical Analysis

The Mann-Whitney U test was used to analyze the number of IgG4-positive cells between the 2 groups. The statistical analysis was performed with GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA); in all cases, $p < 0.05$ was considered statistically significant.

Results

Perforation of tympanic membrane was observed in specimens from all the EOM patients (Fig. 1). Immunohistochemistry revealed a clear presence of IgG4-positive

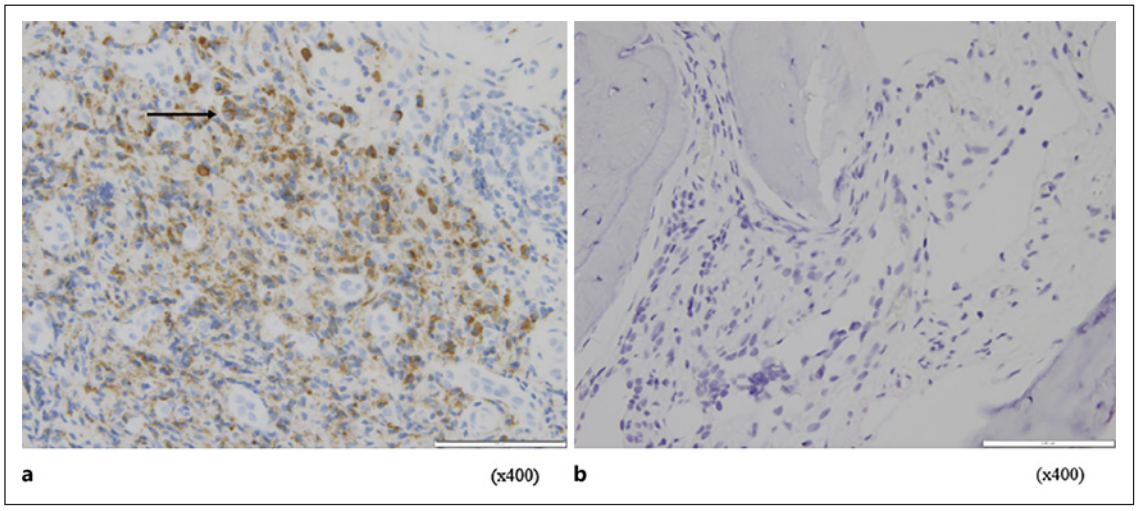

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Fig. 2. a Immunohistochemical findings (arrow: IgG4 staining) of the middle ear mucosa of patients with EOM. A large number of IgG4-positive cells were observed. **b** Immunohistochemical finding of the middle ear mucosa of patients in the non-EOM group. EOM, eosinophilic otitis media.

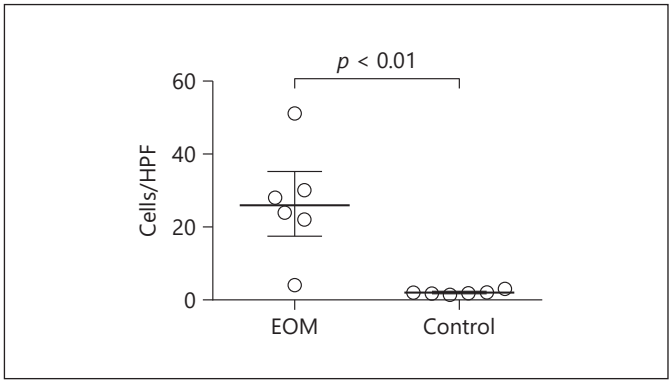

Fig. 3. Comparative analysis of IgG4-positive cells between EOM and the control group. *p* value was determined by Mann-Whitney U test. EOM, eosinophilic otitis media; HPF, high-power field.

Table 2. Patient characteristics ($n = 12$): statistically significant difference was observed in the number of IgG4-positive cells between the patients with EOM and those without EOM

	EOM, $n = 6$	Non-EOM, $n = 6$	*p* value
Age, years	72	71.3	0.892
Sex (W/M)	4/2	3/3	
Hearing threshold, dB	93.5	92.8	0.810
Eosinophil, /μL	388.8	153.7	0.047*
IgG4-positive cell, /HPF	26.5	1.95	0.002*
Serum IgG4, mg/dL	17.9	18.8	0.731

Mann-Whitney U test. * $p < 0.05$. EOM, eosinophilic otitis media; HPF, high-power field.

cells in the middle ear mucosa of these patients (Fig. 2a). The patient characteristics are shown in Table 2. The difference in age and air conduction hearing threshold in the frequencies of 500, 1,000, and 2,000 Hz between the EOM and non-EOM groups was not statistically significant. However, the presence of IgG4-positive cells and the blood eosinophil count between the EOM and non-EOM groups was found to be statistically significant. Statistically significant difference ($p < 0.01$) was also observed in the mean number of IgG4-positive cells between the 2 groups; cell count was higher in patients with EOM – 26.5/HPF than in patients without EOM – 1.95/HPF (Fig. 2a, b, 3). Blood eosinophil counts with EOM (mean [$n = 6$]: 388/μL) are significant higher than those without EOM (mean [$n = 6$]: 153/μL) ($p < 0.05$). The serum IgG4 levels in patients with EOM (mean [$n = 6$]: 17.9 mg/dL) were within the normal limit.

Discussion

EOM pathology has been demonstrated as persistent inflammation of the middle ear along with production of various chemical mediators that induce migration of eosinophils in the mucosa [8]. High concentration of IgE is also detected in the middle ear mucosa; IgE plays a crucial role in type I allergic reaction and mast cell-mediated inflammation. EOM causes marked damage to sensorineu-

ral hearing – nearly 10 times greater loss of BCHL than chronic suppurative otitis media [2]. According to a clinical survey in Japan, 47% of EOM patients show deterioration in BCHL and 6% develop bilateral profound hearing loss [3]. The diagnostic criteria for EOM were proposed in 2011 [9], but the cause of BCHL deterioration in EOM cases remains unclear.

This study showed that the number of IgG4-positive cells in the middle ear mucosa was significantly higher in patients with EOM than in those without EOM. This is the first report to demonstrate the presence of IgG4-positive cells in EOM, and the result is in consensus to that observed in the nasal mucosa in ECRS where the number of IgG4-positive plasma cells is significantly higher in patients with ECRS than without ECRS [7]. Although the role of IgG4 in EOM remains unclear, the results suggest IgG4 participation in EOM pathogenesis.

IgG4 is unique, as it is functionally monovalent and causes little to no inflammation [10]. IgGs normally appear as homodimers, but 2 residues in IgG4 Fc facilitate continuous exchange of monomers, the so-called Fab-arm change [11]. This system leads to recognition of bispecific antigens, which disables its crosslinking capacity and inhibits immune complex formation; together with reduced binding affinity for complement factor C1q, it renders IgG4 as a poor complement activator [12]. Additionally, IgG4 is often considered as a protective blocking antibody (produced after an allergen immunotherapy), as it inhibits or prevents inflammation by competing for antigen binding with the inflammatory IgG subclasses or IgE [13]. Thus, IgG4-positive cells may be induced as a negative regulator to suppress type 2 inflammations in EOM.

Koyama et al. [7] also reported positive correlation between the number of infiltrating IgG4-positive cells in tissues and serum IgG4 levels. In addition, Oka et al. [14] recently showed that serum IgG4 level is higher in patients with moderate to severe ECRS than in those with mild to no ECRS. In the present study, local expression of IgG4 was high in EOM as well as in ECRS.

However, serum IgG4 levels of EOM were within the normal limit; this result was different from the above reports on ECRS [7, 8]. The limitation of the current study is the small sample size; however, pathophysiology of EOM may differ from that of ECRS. Further studies with larger sample size are required to confirm this trend. In addition, it is worthwhile to compare the number of IgG4-positive cells in inflamed middle ear mucosa between EOM and conventional noneosinophilic chronic otitis media.

Conclusions

In the present study, local IgG4 expression was demonstrated in patients with EOM compared to those without middle ear inflammation. This study suggests that IgG4 contributes to the pathogenesis of EOM.

Acknowledgments

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Statement of Ethics

All of the participants provided informed consent. All of the procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration. The International University of Health and Welfare, Mita Hospital Review Board gave the ethical approval (No. 5-18-10) for this study.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Founding Sources

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Author Contributions

Masahiro Takahashi and Mitsuhiro Okano: substantial contributions to the conception or design of this work and analysis of this paper and approval of the final version to be published. Shin Kariya, Atsushi Matsubara, and Satoshi Iwasaki: provide the samples of EOM. Aiko Oka, Yuka Gion, Yasuharu Sato, and Shogo Oyamada: experiment on IgG4 for each sample.

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