Case report

Gene expression of cytokines and prostaglandin metabolism–related proteins in eosinophilic otitis media

Aiko Oka, MD,[a] Masahiro Takahashi, MD,[b] Wataru Nishimura, MD, PhD,[c] Shogo Oyamada, MD,[b] Shinichiro Oka, MD,[b] Satoshi Iwasaki, MD,[b] Kengo Kanai, MD,[a] and Mitsuhiro Okano, MD[a] *Narita and Tokyo, Japan*

The objective of this study was to investigate the levels of gene expression in the middle ear mucosa of 2 patients diagnosed with eosinophilic otitis media. One patient with severe hearing loss showed high expression levels of genes encoding IL-5 and IL-33 receptors. (J Allergy Clin Immunol Global 2024;3:100237.)

Key words: *Eosinophilic otitis media, type 2 inflammation, gene expression, severity, pathogenesis*

Abbreviations used
CRSwNP: Chronic rhinosinusitis with nasal polyps
CS: Corticosteroid
EOM: Eosinophilic otitis media
MEE: Middle ear effusion
PG: Prostaglandin
PGD_2: Prostaglandin D_2
PGE_2: Prostaglandin E_2

Eosinophilic otitis media (EOM) is often accompanied by asthma and chronic rhinosinusitis with nasal polyps (CRSwNP) and is intractable to medication.[1] Patients with EOM experience viscous middle ear effusion (MEE), recurrent infections, and combined sensorineural and conductive hearing loss. Although EOM is rare and nonfatal, frequent visits to the outpatient department and auditory disturbances can impose socioeconomic burdens on patients. The MEE and mucosa of patients with EOM have been reported to show high levels of type 2 cytokine expression and a large number of eosinophils.[2,3] However, cytokine receptors and prostaglandin (PG) metabolism–related proteins that are distinctive to EOM have not been thoroughly investigated. In this study, we examined the gene expression levels of cytokines, PG metabolism–related proteins, and their receptors to elucidate the mechanisms underlying the pathogenesis of EOM.

Two patients diagnosed with eosinophilic otitis media (patients EOM1 and EOM2) according to the diagnostic criteria described by Iino et al[4] were included. In all, 4 patients who underwent cochlear implantation and 1 patient who underwent Vibrant Soundbridge implantation were included as controls (controls 1-5). All of the control subjects were free from middle ear inflammation. Patients who received systemic corticosteroids (CSs) within 1 month or intratympanic CSs/antibiotics within 2 weeks before sample collection were excluded. Blood tests were performed for all patients to assess the eosinophil count and serum IgE levels, and culture tests for otorrhea were conducted for patients with EOM. For the audiometry tests of pure tone averages, the average hearing threshold levels were measured at a set of specified frequencies: 500 Hz, 1000 Hz, and 2000 Hz. This study was performed in accordance with the guidelines of the Declaration of Helsinki, and informed consent was obtained from each patient. The ethics committee of the International University of Health and Welfare Mita Hospital approved this research and the study protocol (approval no. 5-21-38).

A total of 30 genes were analyzed by using a PCR array. The cytokine genes included *IFNG*, *IL10*, *IL13*, *IL17A*, *IL22*, *IL25*, *IL33*, *IL4*, *IL5*, and *TSLP*. The cytokine receptor genes included *CRLF2* (TSLP receptor), *IFNGR1*, *IL10RA*, *IL13RA1*, *IL17RA*, *IL17RB* (IL-25 receptor), *IL1RL1* (ST2 [IL-33 receptor]), *IL22RA1*, *IL4R*, and *IL5RA*. Genes encoding the PG D_2 (PGD_2)/PG E_2 (PGE_2) synthases, namely, *HPGDS* (hematopoietic-type PGD_2 synthase), *PTGES* (microsomal PGE synthase-1 [m-PGES-1] [PGE_2 synthase]), *PTGS1* (COX-1), and *PTGS2* (COX-2) were analyzed. The genes encoding PGD_2/PGE_2 receptors, *GPR44* (CRTH2 [PGD_2 receptor]), *PTGDR* (DP), *PTGER1* (EP1), *PTGER2* (EP2), *PTGER3* (EP3), and *PTGER4* (EP4) were also analyzed. The detailed methods are described in the Supplementary Materials (see the Online Repository at www.jaci-global.org).

RESULTS AND DISCUSSION

The characteristics of the participants in each group are presented in Table I. Both patients with EOM had comorbid asthma, were refractory to treatment, and had eosinophil-dominant MEE.

From [a]the Department of Otorhinolaryngology and [c]the Department of Molecular Biology, International University of Health and Welfare School of Medicine, Narita, and [b]the Department of Otorhinolaryngology, International University of Health and Welfare Mita Hospital, Tokyo.

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Corresponding author: Aiko Oka, MD, 852 Hatakeda, Narita, Chiba Prefecture 286-8520, Japan. E-mail: 19m3019@g.iuhw.ac.jp.

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TABLE I. Clinical characteristics of each sample

Sample	Patient EOM1	Patient with EOM2	Con 1	Con 2	Con 3	Con 4	Con 5
Age (y)	64	58	29	19	47	39	58
Sex (F/M)	F	F	F	F	M	M	F
Blood eosinophil count (no./uL)	290	875	134	8	138	22	34
Serum total IgE level (IU/mL)	18	423					
Comorbidity							
Allergic rhinitis	–	–	–	–	–	–	–
CRSwNP	–	+	–	–	–	–	–
Asthma	+	+	–	–	–	–	–
AERD	+	–	–	–	–	–	–
Atopic dermatitis	–	–	–	–	–	–	–
Endaural findings	COM with simple perforation	COM with perforation and granulation					
Otorrhea culture	*P aeruginosa* 1+	*S aureus* 3+					
Audiogram (air R/L, dB)	97.5/SO	45/15					
Audiogram (bone R/L, dB)	105/SO	36/15					
Medication	Oral anti-LT	Nasal CS spray	–	–	–	–	–

AERD, Aspirin-exacerbated respiratory disease; *COM*, chronic otitis media; *Con*, control; *F,* Female; *L*, left; *LT*, leukotriene *M,* male; *R*, right; *SO*, scale out.

In addition, patient EOM1 had aspirin-exacerbated respiratory disease, and patient EOM2 had CRSwNP. Patient EOM1 had perforations in both tympanic membranes (Fig 1, *A*), severe bilateral sensorineural hearing loss, and *Pseudomonas aeruginosa* infection; was taking oral anti-leukotriene medication; and had a disease duration of 15 years. Patient EOM2 had granulation in both middle ear spaces (Fig 1, *B*), mild mixed hearing loss in the right ear, and a *Staphylococcus aureus* infection; was using nasal CS spray; and had a disease duration of 11 years. None of the patients in the control group were taking any medications.

Although we used RNA samples with an A260/A280 ratio greater than 1.8, the expression of some genes could not be detected. *IL4*, *IL22*, and *IL25* were excluded because their expression levels in the samples from 1 or both patients with EOM were below the limit of detection. *IL17A* was also excluded because all expression levels from the control samples were below the limit of detection; therefore, there were no usable values for calibration. Consequently, we chose the sample from control patient 4 for calibrating the ΔΔCt method because that sample did not have any missing values after these exclusions. Fig 2 shows a heatmap of the gene expression levels for each sample. Red indicates high gene expression levels, and green indicates low gene expression levels. Patient EOM1 appeared to be different from all of the other patients, whereas patient EOM2 appeared to be similar to all of the control patients.

The expression levels of *IL1RL1* and *IL5RA* were higher in patient EOM1 than in patient EOM2 and the control patients (Fig 3). *IL1RL1* encodes the receptor for IL-33 and is expressed on the membranes of various immune cells, such as T_H2 cells, regulatory T cells, type 2 innate lymphoid cells, macrophages, mast cells, eosinophils, basophils, and neutrophils. IL-33 is released mainly from epithelial cells in response to stress or damage. Immune cells are activated via ST2/IL-33 signaling, leading to a type 2 immune response.[5] *IL5RA* encodes the receptor for IL-5, which plays an essential role in eosinophil differentiation, induction, and proliferation. IL-5 signaling in eosinophils induces chemotaxis and activation of integrin CD11b, and it prolongs eosinophil survival by inhibiting apoptosis.[6]

In contrast, the expression levels of the genes *IFNG*, *IFNGR1*, *IL10RA*, *PTGES*, and *PTGS1* were low in both patients with EOM (Fig 3). In addition, the level of expression of the gene *IL10* was lower in patient EOM1 than in patient EOM2 and the control patients (Fig 3). IFN-γ is produced by T_H1 cells, natural killer T cells, macrophages, and natural killer cells and is associated mainly with antiviral and antimicrobial responses.[7] IL-10 is secreted by various immune cells in response to inflammation and it counterregulates both innate and acquired immune responses.[8] In the present study, the severe hearing loss in patient EOM1 and granulation formation in patient EOM2 could be associated with bacterial infections. *P aeruginosa* infection is associated with a high risk of developing sensorineural hearing loss.[9] The presence of bacterial infections was consistent with the findings of a previous study showing that both eosinophils and neutrophils are likely involved in middle ear inflammation associated with patient EOM.[2] *PTGES* encodes microsomal prostaglandin E synthase-1, the main terminal synthase of PGE_2 in the airways. PGE_2 is known to attenuate the migration of eosinophils and suppress the release of type 2 cytokines, and it exhibits low expression levels in patients with asthma and CRSwNP,[10,11] the pathogenesis of which also involves an eosinophilic mechanism. The gene functions to suppress type 2 inflammation and its low expression level may be associated with type 2 inflammation. *PTGS1* encodes COX-1, which is constitutively expressed under physiologic conditions, whereas COX-2 is expressed during inflammation and injury.[12] The number of COX-1–positive cells in nasal polyps has been reported to be reduced by the application of topical CSs.[13] Patient EOM1 had previously used CS ear drops, and patient EOM2 had used a nasal CS at the time of sample collection. The other genes showed no clear trends (data not shown).

Although we had samples from only 2 patients with EOM, each patient had different clinical and RNA expression characteristics. Patient EOM1 had a simple perforation of the tympanic membrane with a low blood eosinophil count and serum IgE level, despite having had severe hearing loss with increased expression levels of genes encoding IL-5 and IL-33 receptors. In contrast, patient EOM2 showed granulation with a

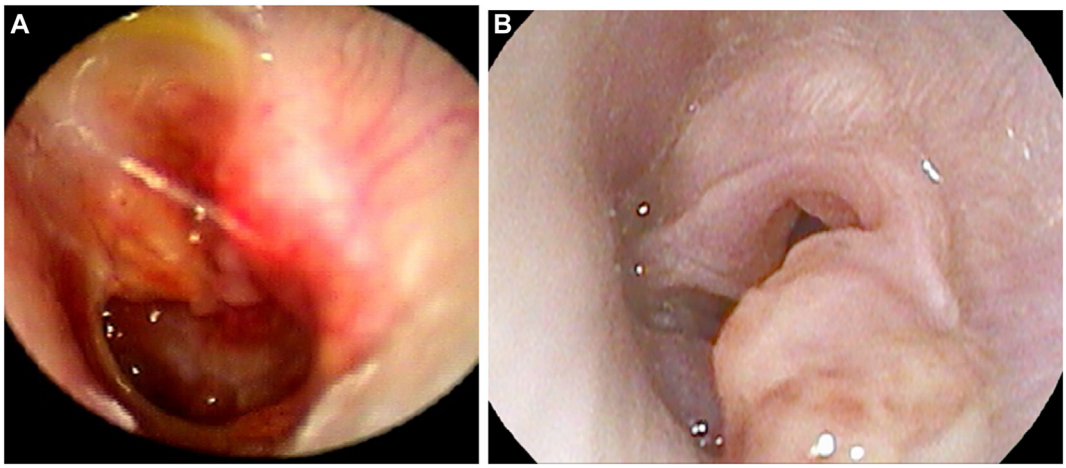

FIG 1. Endaural findings in left ears of patients EOM1 (**A**) and EOM2 (**B**).

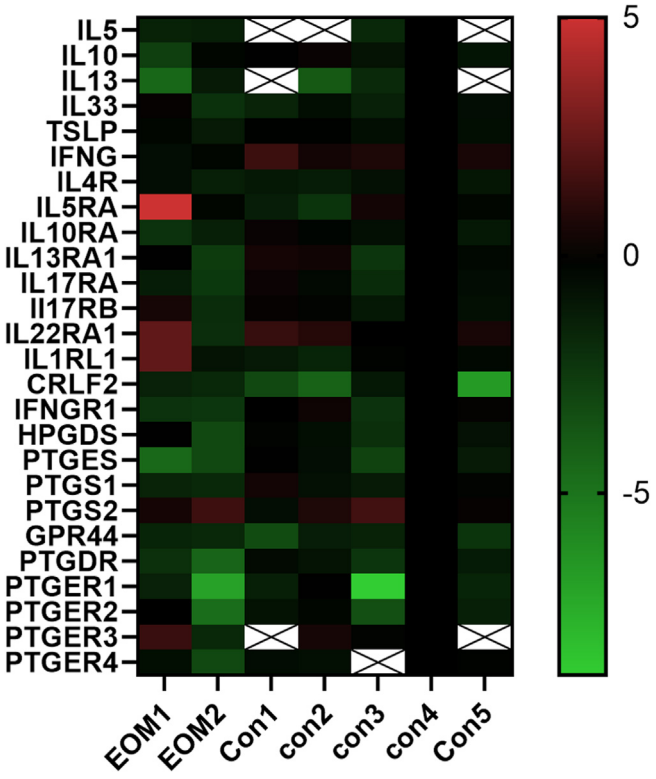

FIG 2. Heatmap of the gene expression levels for each sample. Red indicates high gene expression levels, and green indicates low gene expression levels.

high blood eosinophil count and serum IgE level but did have mild hearing loss and gene expression levels similar to the control patients. Our 2 case patients demonstrate that mild endaural findings, as well as a low blood eosinophil count and IgE level in patients with EOM, do not always indicate mild middle and/or inner ear inflammation.

The present study has some limitations, including a small sample size, local CS treatment in patients with EOM, and a lack of samples from patients with chronic suppurative otitis media. Biopsy is an invasive method that cannot be adapted for all patients with EOM. Samples from the middle ear mucosa of patients with EOM were collected to exclude the possibility of tumors or vasculitis clinically but not specifically for this study or the diagnosis of EOM, because we used the major diagnostic criterion of eosinophil-dominant middle ear effusion. In addition to the low prevalence of EOM, the invasiveness of biopsy was another reason for the small number of samples in the present study. In addition, future studies to clarify the mechanisms of EOM, including studies investigating other molecules such as Toll-like receptors, are desired, especially when inflammation may be related to infection, as in the patients with EOM in this study. Although patients who received systemic CS within 1 month before sample collection were excluded from this study, past systemic CS use may have also affected the results. The low blood eosinophil count and IgE level in patient EOM1 may have been affected by systemic CS administered 6 months before the biopsy. Future clinical studies with larger sample sizes, restrictions on CS treatment (including nasal spray) before sample collection, and samples from patients with chronic suppurative otitis media are required.

In conclusion, the sample of the middle ear mucosa of the patient with EOM and severe hearing loss showed high levels of expression of genes encoding IL-5 and IL-33 receptors. In addition, the samples from both patients with EOM showed low levels of expression of genes that encode IFN-γ, IL-10, PGE_2, and COX-1. Even in patients in whom EOM appears to be mild, the levels of expression of inflammation-related genes may be high, and the possibility of inner ear damage should be considered. Future studies with larger sample sizes are needed to confirm these findings.

DISCLOSURE STATEMENT

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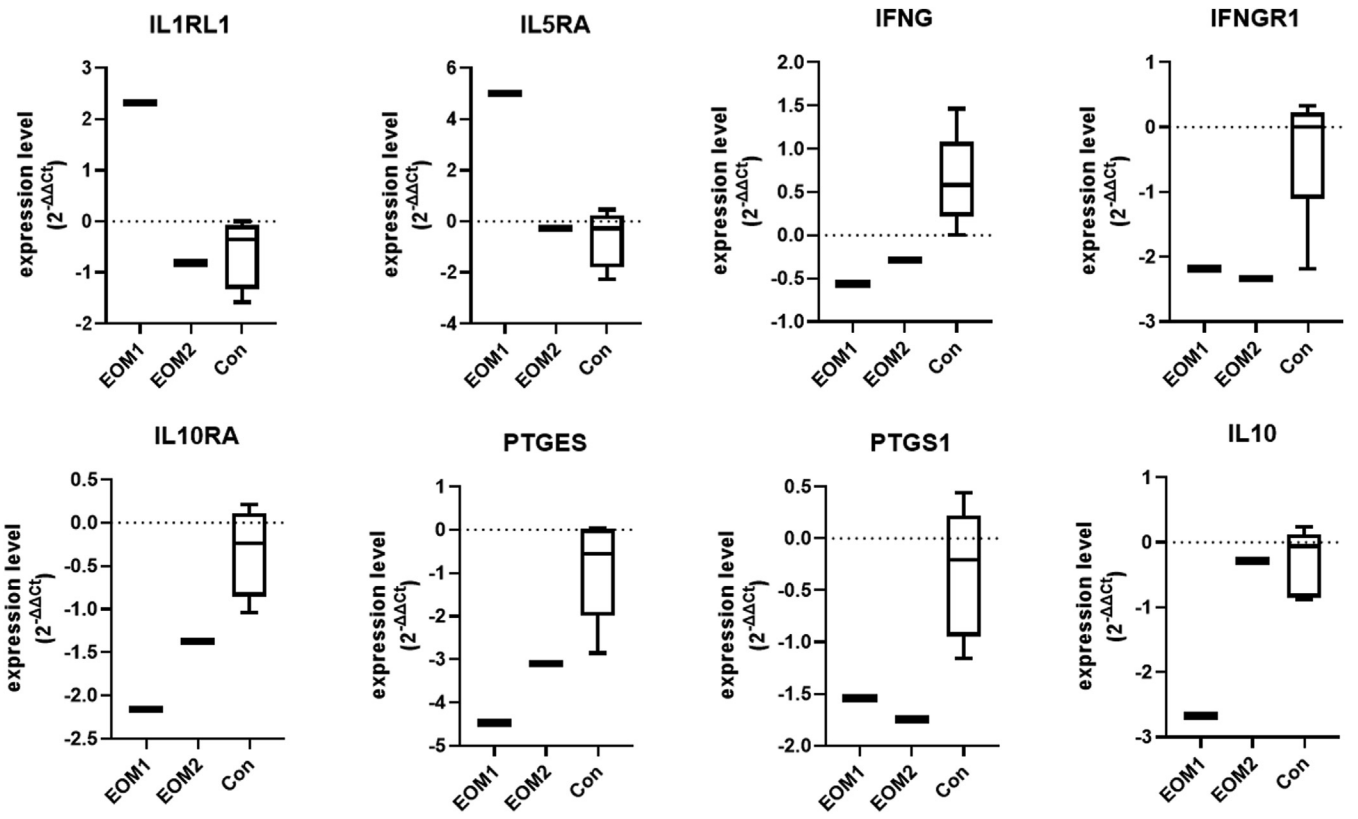

FIG 3. Gene expression levels of *IL1RL1*, *IL5RA, IFNG, IFNGR1, IL10, IL10RA, PTGES,* and *PTGS1* in the patients with EOM versus in the control patients.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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