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Original article

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

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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-1β, MIP-1α, G-CSF, IL-1ra, IL-4, IFN-γ, MIP-1β, 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-1ra, IL-2, IL-5, IL-9, IL-13, eotaxin, MIP-1α, PDGF-BB, and RANTES in the EOM group ($p < 0.05$).
Conclusions: Our study showed that IL-5, IL-2, MIP-1α, and IL-1ra are the important factors involved in EOM pathogenesis. Furthermore, not only eosinophil, but also neutrophil are involved in middle ear inflammation of EOM.

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

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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-1α (MIP-1α), 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, END, 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-1β, 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-1α, MIP-1β, 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 mem-