higher in the former group, which is a finding that conflicts with our hypothesis. One possibility is that viral inflammation was not reflected by the changes in the ATA group. There might be other ways in which CXCL10 contributes to viral-induced exacerbation of CRS. For instance, CXCL10 upregulates eosinophil functions such as adhesion and O_2 generation and also increases the release of eosinophil-derived neurotoxin when eosinophilic infiltration occurs during the exacerbation of asthma.[42] CXCL10 also has a prominent role in the worsening of airflow obstruction and airway inflammation in patients with acute rhinovirus-induced asthma.[34] Some recent studies have suggested that CXCR3 may be expressed by human CD25hi FOXP3+ CD4+ Tregs, a T cell subset with potent immunoregulatory properties,[43] which suggests a paradoxical role for CXCL10. The exact functional implications of these findings can only be explained by further investigation.

The pathogenesis of ATA and AIA is different. AIA is attributable to inhibition of cyclooxygenase by aspirin-like drugs and does not arise from an allergic reaction. Biosynthesis of cysteinyl leukotrienes is also upregulated in patients with AIA.[44] However, both AIA and ATA are associated with eosinophilic sinusitis and nasal polyposis. The clinical impact of ATA and AIA on CRS may be influenced by many factors, but the differences between ATA and AIA have not been well documented. Basement membrane hyperplasia, goblet cell proliferation, and eosinophil infiltration have been reported to be more prominent in the nasal polyps of asthma patients than in polyps from patients without asthma.[45] Based on our findings in the present study, diseases of the lower airways such as ATA and AIA seem to influence gene expression in nasal polyp fibroblasts, suggesting that concomitant lower airway disease is a major reason why CRS may become refractory to treatment.

In conclusion, we found that CXCL10 expression was upregulated by Poly I:C stimulation in nasal fibroblasts from CRS patients with asthma and this induced Th1 cell infiltration into nasal polyp tissues. Although the mechanism leading to differences of CXCL10 expression between CRS patients with or without asthma needs to be clarified, our findings suggest that CRS associated with asthma may become intractable due to the overproduction of CXCL10 in response to viral infection.

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The Expression of Protease-Activated Receptors in Chronic Rhinosinusitis

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Key Words
Protease-activated receptors · Eosinophils · Asthma · Chronic rhinosinusitis

Abstract
Background: A recent study suggested that protease-activated receptors (PARs) are involved in allergic respiratory diseases, such as asthma. Chronic rhinosinusitis (CRS) is one of the most common chronic airway diseases, but little is understood about its pathogenesis. The purpose of this study was to compare the expression and distribution of PARs in biopsy specimens obtained from CRS and control patients. ***Methods:*** Biopsy specimens were obtained from 7 pituitary tumor patients as controls, 8 CRS patients with aspirin-tolerant asthma (ATA), 7 CRS patients with aspirin-induced asthma (AIA), and 7 CRS patients without asthma (CRS). Sections were stained for PAR-1, PAR-2, PAR-3 and PAR-4 using specific polyclonal antibodies. Staining was scored semiquantitatively for both intensity and distribution. To confirm the presence of PARs on inflammatory cells, double staining with eosinophil cationic protein (EG2) and elastase was also performed. ***Results:*** Both the epithelium and the infiltrating inflammatory cells in the CRS with asthma groups showed significant upregulation of the expression of PAR-2 and PAR-3 compared with the CRS without asthma group and the control group. In the patients with CRS complicated by asthma, eosinophils were increased among PAR-2- and PAR-3-positive cells. In the patients with CRS not complicated by asthma, neutrophils were increased among PAR-2-positive cells. ***Conclusions:*** Differences in the expression of PAR-2 and PAR-3 on epithelial cells, eosinophils and neutrophils may be involved in the pathogenesis of CRS. CRS may be able to be treated by targeting PAR-2 and PAR-3.

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Introduction

Chronic rhinosinusitis (CRS) is one of the most frequent chronic diseases in the USA. The National Center for Health Statistics has described the increasingly expensive health care burden that CRS inflicts in the USA; with an estimated 18–22 million cases, CRS is one of the main chronic diseases [1]. CRS is characterized by inflammatory mucosal thickening and polyp formation in the paranasal sinuses. Histological studies have demonstrated that the inflammation typically involves accumulation of activated eosinophils in the sinus mucosa and submucosa [2]. These eosinophils are considered to play a major role in the pathogenesis of CRS via release of their granules, which contain toxic proteins such as eosinophil cationic protein and major basic protein [2–4]. The association between sinusitis and asthma, especially severe asthma,

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has long been noted. A report in 1980 described a high prevalence of abnormal sinus mucosa in asthma patients [5]. The prevalence of asthma in patients with CRS has been reported to be as high as 50% [6]. Hansel [7] called attention to the histopathological similarity between nasal, sinus and bronchial tissues in subjects with asthma, with the most outstanding feature being their infiltration by eosinophils. In all these tissues, the pathological findings, local eosinophilia and thickening of the mucosa and epithelial basement membrane and hyperplasia of goblet cells and gland cells can be seen. Extreme peripheral eosinophilia (i.e. a count of 520/μl or more) and asthma place patients at a high risk for recurrence of CRS within 5 years after surgery [8]. Although asthma and CRS have many similarities, we still know little about the factors causing chronic immune activation and persistent eosinophilic inflammation in both diseases.

Protease-activated receptors (PARs) are G protein-coupled receptors that are stimulated by proteases. When proteases cleave the N-terminus of PARs, the new N-terminus of this receptor attaches to the second loop of the extra terminal. The receptor is then activated. PAR-1, PAR-2, PAR-3 and PAR-4 have been identified so far, and PARs are widely expressed on vascular cells, connective tissue cells, leukocytes, epithelial cells and many airway cells [9]. Several reports implicate a role for PARs, especially PAR-2, in airway inflammation and asthma. In mouse airways in vivo, coadministration of PAR-2 agonist peptide and an experimental antigen, ovalbumin (OVA), enhanced Th2-type sensitization to OVA, while administration of OVA alone induced tolerance [10]. The eosinophil count in the bronchoalveolar lavage was significantly increased in PAR-2 transgenic mice, but it was significantly decreased in PAR-2 knockout mice [11]. In human epithelial cells, PAR-2 recognizes serine protease allergens, such as Der p 3, Der p 9 and Pen c 13 as well as arginine-specific (trypsin-like) cysteine proteinases, and aspartate protease from a fungus, *Alternaria*, induces the production of proinflammatory cytokines and chemokines [12–16]. Although human eosinophils express PAR-2 and PAR-3 mRNA, only PAR-2 works functionally [17]. Stimulation of PAR-2 on human eosinophils results in superoxide production and degranulation [17]. Recently, we found that aspartate protease from *Alternaria* induces activation and degranulation of human eosinophils that are mediated by PAR-2 [18–20]. In patients with asthma, PAR-2 was overexpressed in airway epithelial cells [21], but PAR-1, PAR-3 and PAR-4 were not increased. These findings indicate that PAR-2 is involved in the pathogenesis of asthma; although we know little about the functions of PARs in CRS, we hypothesized that they are indeed involved in its pathogenesis. We investigated PAR expression in nasal polyps and the sinus mucosa of CRS patients and healthy volunteers. Both the epithelium and the infiltrating inflammatory cells in the CRS with asthma groups showed a significant upregulation of expression of PAR-2 and PAR-3 when compared with the CRS without asthma group and the control group. In the patients with CRS that was not complicated by asthma, neutrophils were increased among PAR-2-positive cells. In the CRS with asthma groups, eosinophils were increased among PAR-2- and PAR-3-positive cells. Our findings indicate that differences in the expression of PAR-2 and PAR-3 on epithelial cells, eosinophils and neutrophils may be involved in the pathogenesis of CRS.

Table 1. Background data of the subjects

	Number	Sex M:F	Age years	Airway allergic disease	Peripheral eosinophils %
Healthy controls	7	5:2	48±14.3	none	3±2.3
CRS	7	6:1	49.4±13.0	none	2.3±3.9
CRS ATA	8	5:3	54.6±12.4	ATA	10.3±6.7*
CRS AIA	7	4:3	50±13.1	AIA	7.0±5.8*

* $p < 0.05$ vs. CRS, healthy controls. Differences between CRS groups were tested using one-way ANOVA.

Materials and Methods

Materials

Nasal sinus mucosal specimens were obtained from patients who had been diagnosed with CRS and had undergone endoscopic nasal surgery in the Department of Otorhinolaryngology of the Jikei University School of Medicine. The CRS patients were classified into 3 groups on the basis of the presence or absence of asthma as a complication: CRS patients (n = 7) with aspirin-tolerant asthma (CRS ATA), CRS patients (n = 8) with aspirin-induced asthma (CRS AIA) and CRS patients (n = 7) without asthma (CRS). In addition, as a control, nasal sinus mucosal specimens were obtained at the time of transnasal surgery for pituitary tumors in patients (n = 7) who had no nasal sinus inflammation. Table 1 shows the background data for the 29 patients who comprised the above 4 groups.

Reagents

Anti-PAR-1, anti-PAR-2, anti-PAR-3 and anti-PAR-4 antibodies (rabbit polyclonal anti-human) were obtained from Gene Tex Inc. (San Antonio, Tex., USA), while anti-rabbit IgG antibody

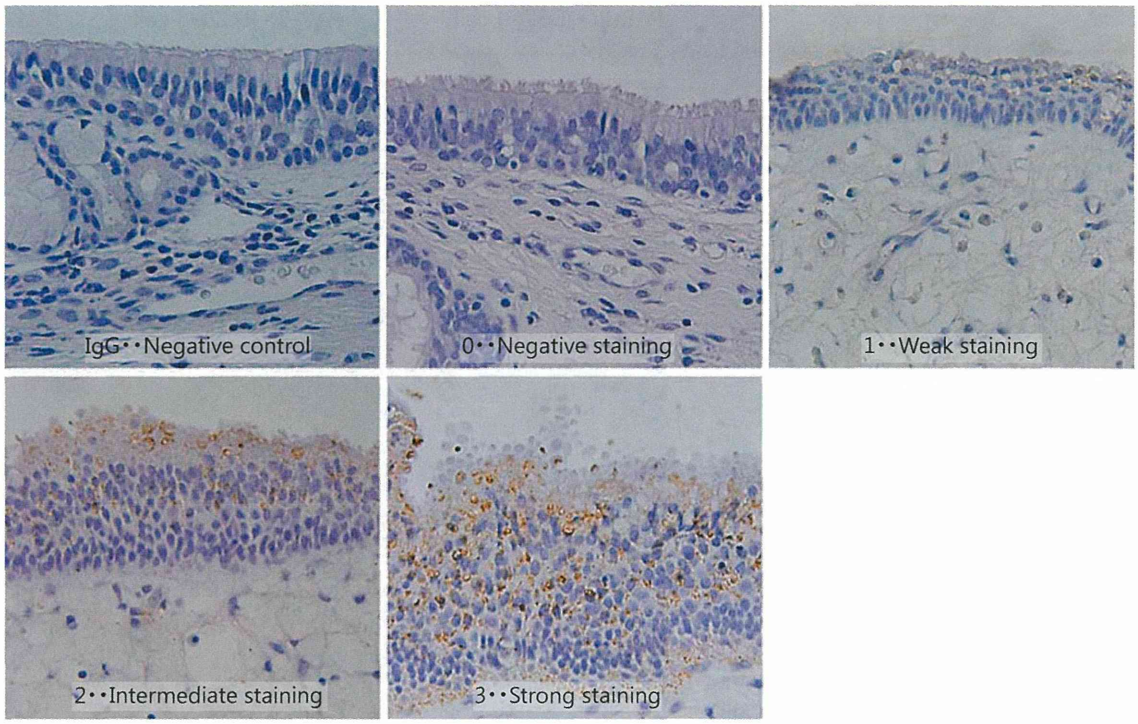

Fig. 1. Assessment of epithelial staining. The intensity of staining was evaluated using scores from 0 to 3, in which 0 was the intensity of staining with IgG as the negative control. The figure shows representative examples of IgG, negative staining, weak staining, intermediate staining and strong staining.

was purchased from BD Pharmingen, (Franklin Lakes, N.J., USA). DakoCytomation ENVISION kit/HRP rabbit antibody, DakoCytomation ENVISION-labeled polymer-AP mouse/rabbit antibody, and anti-human neutrophil elastase antibody were purchased from Dako (Copenhagen, Denmark). Monoclonal antibodies against human eosinophil cationic protein (EG2) were obtained from Pharmacia (Uppsala, Sweden).

Immunohistochemistry

The sinus mucosal and nasal polyp specimens obtained during surgery were – in the operating theater – fixed in 10% formalin solution, dehydrated with 100% alcohol and then embedded in paraffin. The specimens in paraffin-embedded blocks were separated by a width of 3 μm on slide glasses and sectioned. The sections were then deparaffinized with xylol followed by alcohol. The deparaffinized sections were placed in 1% BSA for 5 min at room temperature to block endogenous peroxidases. The sections were then washed in TBS (Tris-buffered saline, Dako). The slides were incubated with primary polyclonal anti-PAR-1, anti-PAR-2, anti-PAR-3 and anti-PAR-4 IgG antibodies as a negative control, for 120 min at room temperature. The sections were washed with TBS and then reacted with ENVISION kit/HRP rabbit antibody for 1 h at room temperature. The sections were again washed with TBS, followed by color development using peroxidase, with DAB as the chromogenic substrate. In addition, immunostaining for PAR-2 and PAR-3 was performed, followed by double immunostaining using monoclonal antibodies against human EG2 and anti-human neutrophil elastase antibody. Each staining reaction was allowed to proceed for 1 h at room temperature. The sections were then washed with TBS and reacted with DakoCytomation ENVISION-labeled polymer-AP mouse/rabbit antibody for 1 h at room temperature. After washing again with TBS, color development was performed using Fast Red (Roche Diagnostics, Indianapolis, Ind., USA).

Evaluation of Staining

We evaluated the epithelial cells and the subcutaneous inflammatory cells in the specimens separately. Staining of the epithelium for PARs was scored by 3 independent investigators blinded to the treatment status of the individual donors. Briefly, as described previously [12], the intensity of staining of the epithelium in 2 low-power fields was assessed in comparison with the negative control, which had been stained with IgG. Scores from 0 to 3 were used to grade the intensity (fig. 1). The total score was employed as the staining score.

To determine the infiltrating inflammatory cell count, cells that stained positively with anti-PAR-1, anti-PAR-2, anti-PAR-3 and anti-PAR-4 antibodies in a ×400 high-power field were counted. In addition, in order to eliminate differences in the infiltrating inflammatory cell count due to variation among individuals, the total cell count in the same field was determined, and the PAR-positive rate was calculated. Moreover, to determine which inflam-

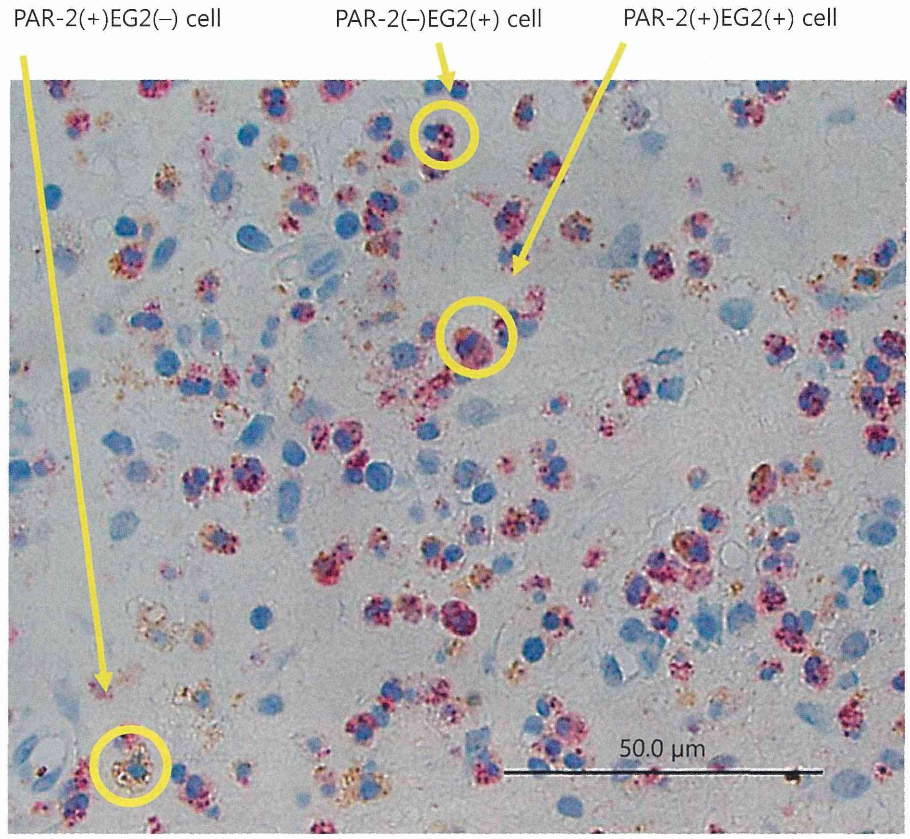

Fig. 2. The results of double immunostaining of a polyp specimen from a CRS ATA patient using anti-EG2 antibody and anti-PAR-2 antibody. Granules in eosinophils are stained red in the presence of EG2, whereas cells that stain positively with anti-PAR-2 antibody appear brown.

matory cells were positive for PAR-2 and PAR-3, we performed double immunostaining of the cells with anti-ECP and anti-elastase antibodies and counted the cells that were positive for both markers (fig. 2).

Statistics

Differences between CRS groups were tested using one-way ANOVA and Tukey's honestly significant difference test. $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 16.0J software (Chicago, Ill., USA).

This study was approved by the ethics committee of the Jikei University Hospital, Tokyo, Japan.

Results

Patients' Background

Table 1 shows the background data for the study patients. There were no statistically significant differences among the 4 groups with regard to number, gender or age of patients. However, the numbers of peripheral eosinophils in the CRS ATA group and the CRS AIA group were significantly higher than in the other 2 groups.

PAR Expression in Epithelial Staining

There were no statistically significant differences among the 3 CRS groups with regard to the surface expression of PAR-1 and PAR-4 when the epithelial cells were stained (fig. 3). However, the expression levels of PAR-2 and PAR-3 were significantly upregulated in the CRS ATA and CRS AIA groups when compared with the control group.

PAR Expression on Infiltrating Inflammatory Cells

We investigated PAR expression on subepithelial inflammatory cells. The PAR-1-positive rate was less than 50% in each of the patient groups, and there were no statistically significant differences among the control, CRS and CRS ATA groups. Only the CRS AIA group showed a statistically significant difference versus the control group. The PAR-2-positive rate was significantly different in both the CRS ATA and CRS AIA groups compared with the control group and the CRS group. The PAR-3-positive rate was significantly higher in both the CRS ATA and CRS AIA groups compared with the control group.

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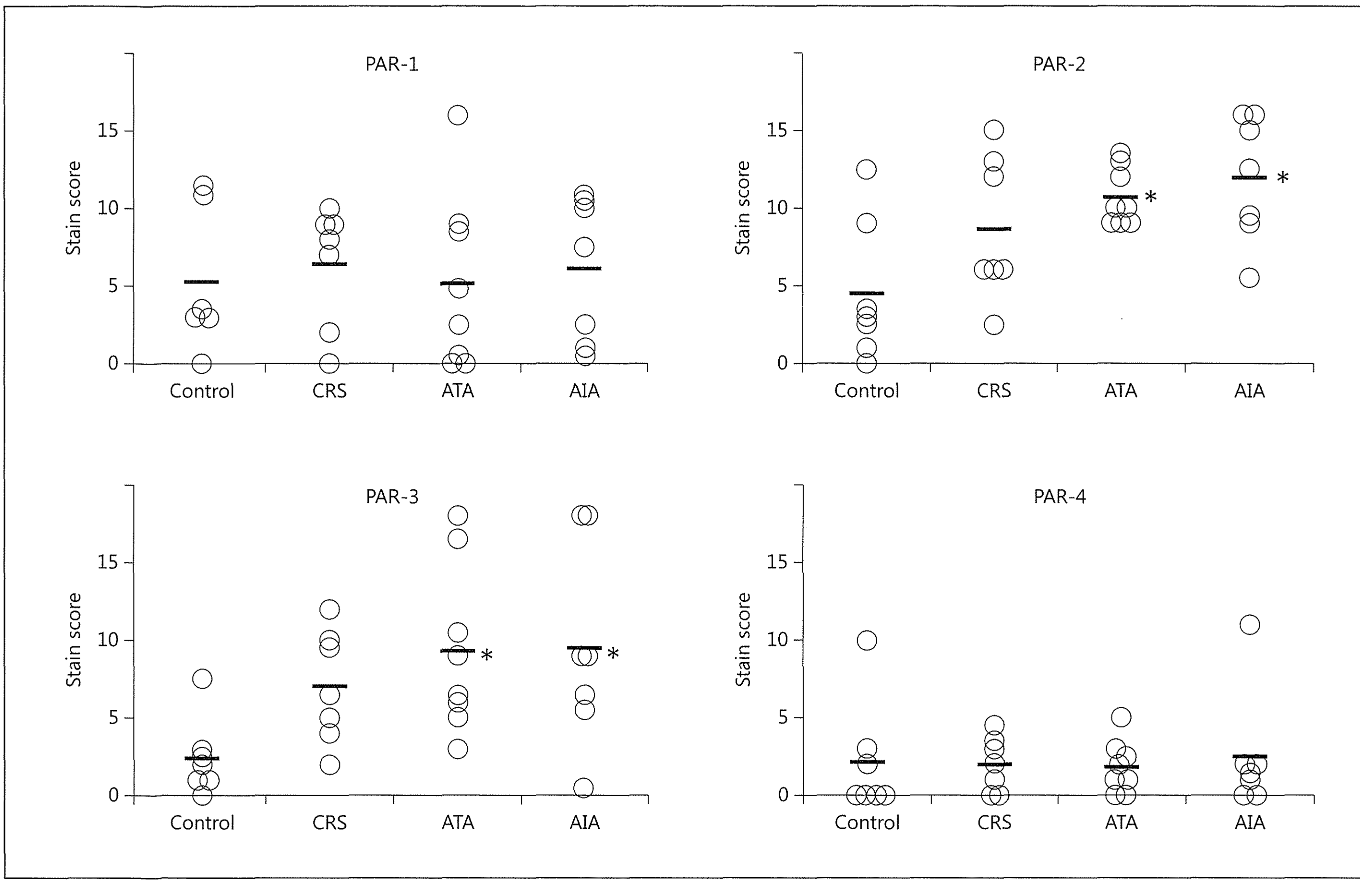

Fig. 3. Immunohistochemical scoring of PARs in the epithelium. The y-axis shows the staining score, with a minimum of 0 and a maximum of 18. The x-axis shows each of the subject groups. The score itself is shown as a small circle, and the bar shows the mean score for the group. * $p < 0.05$ compared with the control group.

The PAR-4-positive rate did not differ significantly among the 4 patient groups. The positive rates for PAR-2 and PAR-3 on the inflammatory cells in the sinus mucosa of the CRS patients were significantly increased by the presence of asthma as a complication, especially when ATA or AIA was present (fig. 4).

Double Staining of PAR-2-Positive and PAR-3-Positive Cells for EG2 and Elastase

The percentage of PAR-2-positive cells that stained positively for EG2 was significantly higher in each of the CRS groups compared with the control group. Moreover, the percentage was significantly higher in the asthma-complicated groups than in the CRS group. In the double staining for PAR-2 and elastase, the positive rate in the CRS group was markedly higher than in the other 3 groups. In the CRS ATA, CRS AIA and control groups, there were almost no cells showing double-positive staining. The staining results for PAR-3 were almost the same as those for PAR-2. The percentage of PAR-3-positive cells that stained positively for EG2 was significantly higher in each of the CRS groups than in the control group, and even in the asthma-complicated groups compared with the CRS group. However, although the results of double staining for PAR-3 and elastase showed a similar tendency, there were no statistically significant differences among the disease groups (fig. 5).

Next, we investigated the positive rate for double staining of EG2-positive cells for PAR-2 and PAR-3 (fig. 6). Compared with the control group, the CRS groups each showed significantly higher rates of expression of PAR-2 and PAR-3 on the EG2-positive cells. Moreover, approximately 90% of the EG2-positive cells in the CRS ATA and CRS AIA groups expressed PAR-2 and PAR-3, and the

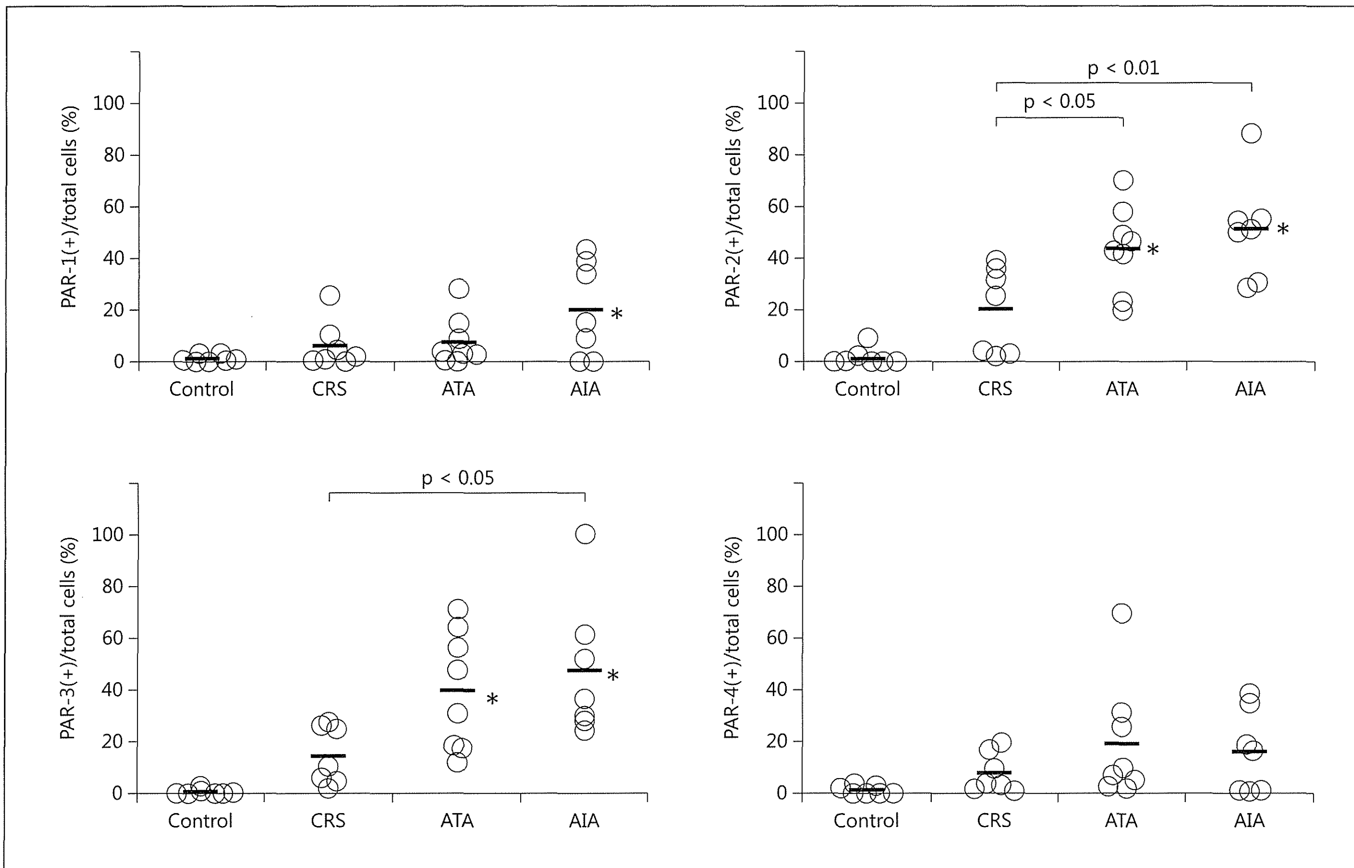

Fig. 4. PAR expression by infiltrating cells. The y-axis shows the positive rate for PARs in the infiltrating inflammatory cell population. Expression of PAR-2 was significantly increased in the CRS ATA and CRS AIA groups compared with the control and CRS groups. Expression of PAR-3 was significantly increased in the CRS ATA and CRS AIA groups. The x-axis shows each of the subject groups. The score itself is shown as a small circle, and the bar shows the mean score for the group. * $p < 0.05$ compared with the control group.

positive rates were significantly higher than in the CRS group. The results thus showed that most of the cells infiltrating the submucosa of the nasal sinus of CRS patients with a complication of asthma express PAR-2 and PAR-3.

Discussion

This study generated various findings regarding the expression of PARs on the nasal sinus mucosa of CRS patients. We showed that the epithelium and infiltrating inflammatory cells in the CRS with ATA and AIA groups had significant upregulation of expression of PAR-2 and PAR-3 in comparison to the CRS without asthma groups and the control group. Previous reports support our data. For example, PAR-2 mRNA expression was significantly greater in tissues from patients with acute rhinosinusitis, CRS and nasal polyps compared with control tissues from healthy sinus subjects [22]. Furthermore, anti-PAR-2 immunostaining of the surface epithelium of nasal polyps and conjunctival epithelium was significantly greater than the control [22, 23]. The human respiratory epithelium is the first interface of contact with airborne pathogens and allergens. Upon activation, epithelial cells produce antimicrobial molecules, proinflammatory cytokines and chemokines for the recruitment of immune cells to the local airway via pattern recognition receptors (PRRs). PRRs recognize conserved structural motifs expressed by microbial pathogens or pathogen-associated molecular patterns (PAMPs) [24–26]. Among the PRRs, PARs are widely distributed on the cells of the airways, where they contribute to the inflammation characteristic of allergic

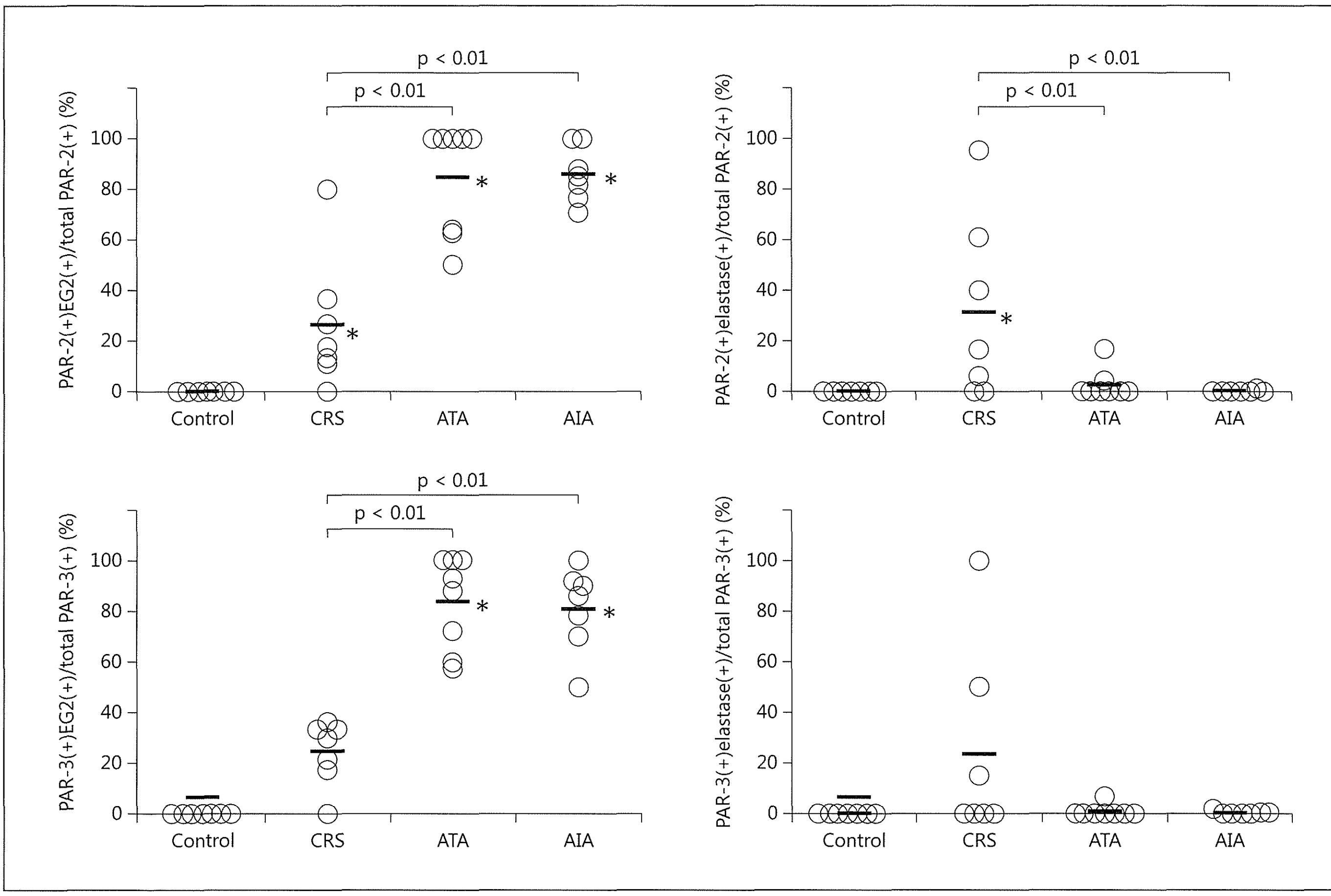

Fig. 5. The results of double immunostaining, showing the double-positive cells per PAR-2- or PAR-3-positive cells (in percent). The y-axis shows the positive rate for EGS and elastase in PAR-2- or PAR-3-positive cells. The x-axis shows each of the subject groups. The score itself is shown as a small circle, and the bar shows the mean score for the group. * $p < 0.05$ compared with the control group.

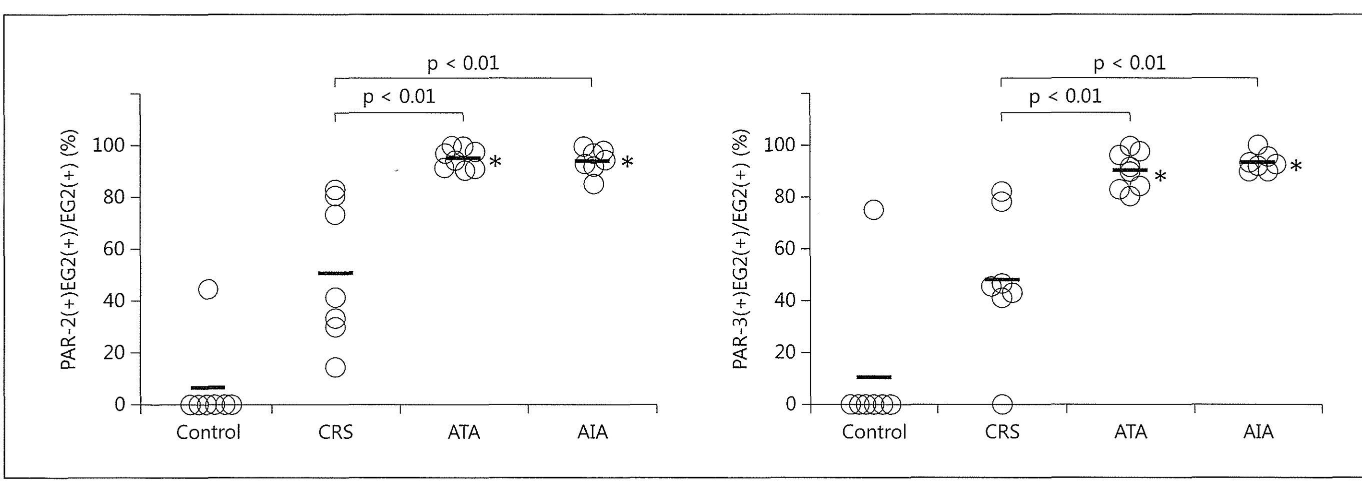

Fig. 6. The results of double immunostaining, showing the PAR-2- or PAR-3-positive cells per EG2-positive cells (in percent). * $p < 0.05$ compared with the control group.

diseases [27]. PAR stimulation on epithelial cells opens tight junctions, causes desquamation and leads to production of cytokines, chemokines and growth factors [21, 27]. PAR-2, apparently the most important of the 4 PARs that have been characterized to date, is increased on the epithelial cells of patients with asthma [21] and allergic rhinitis [28]. Cysteine protease from *Alternaria* induces epithelial cell thymic stromal lymphopoietin production via PAR-2 in vitro [29]. Thymic stromal lymphopoietin is thought to be a necessary cytokine for the development of Th2-type airway inflammation [30, 31]. Furthermore, PAR-2-mediated recognition of aspartate protease activity that is secreted by actively growing *Alternaria* triggers human epithelial cells to become activated and produce cytokines [16]. On the basis of these various findings, it can be thought that PAR-2 is activated by proteases produced by the airway microbiome, and this is one of the natural immune responses that cause allergic inflammation. We still know little regarding PAR-3, but our findings suggest that it may indeed be involved in the pathophysiology of CRS, a concept that we hope to investigate in the future. When we look at the infiltrating inflammatory cells, we see that the composition of cells that express PAR-2 and PAR-3 differs as a function of whether or not CRS is complicated by asthma. That is to say, in the CRS groups with a complication of ATA or AIA, eosinophils made up nearly 80% of the cells that expressed PAR-2. In contrast, in the CRS group with no complication of asthma, eosinophils comprised only about 30% of the PAR-2-expressing cells. Similar results were obtained in regard to PAR-3 expression. If we focus on cells that stained positively with anti-EG2 antibody, statistically significant differences in the rates of expression of PAR-2 and PAR-3 are seen as a function of the presence or absence of asthma as a complication. These results demonstrate that even within the scope of the same disease, CRS, the expression of receptors on eosinophils that have infiltrated the airway submucosa is altered by whether or not asthma is present as a complication. Previous reports support our data, i.e. the number of eosinophils expressing PAR-2 was significantly elevated even in the nasal mucosa of seasonal allergic rhinitis compared with the controls [28]. Based on our data, human eosinophils are activated by live *Alternaria alternata* organisms, release their granule proteins and kill the fungi. Eosinophils, but not neutrophils, responded to products secreted by *A. alternata* [20]. We also found that eosinophils are equipped with innate cellular activation machinery that responds to the extracellular aspartate protease activity secreted by *Alternaria* [19] and to cockroach extracts [32, 33]. A novel mechanism is likely involved in activation of PAR-2 compared to serine protease activation of PAR-2 [19]. Thus, human eosinophils may recognize certain danger signals or virulence factors produced by fungi and then provoke inflammatory responses against these organisms. Dysregulation of such an innate immune mechanism may be involved in the pathophysiology of human diseases such as asthma and CRS [18]. In addition, it is interesting that potentiation of PAR expression on the surface of eosinophils was seen in the CRS groups with a complication of asthma, a disease that manifests in repeated bouts. It is thought that one reason that CRS complicated by asthma readily becomes intractable is that the absolute number of eosinophils releasing various cytotoxic granules is large [8]. Moreover, it can be thought that existing inflammation is exacerbated by stimulation of the PAR-2 and PAR-3 receptors that are expressed at high rates on eosinophils. Also, with regard to receptor expression on neutrophils, almost no expression of PAR-2 and PAR-3 receptors was seen in the CRS groups with a complication of asthma, and high levels of expression were found only in the asthma-free CRS group. These differences in the expression of PAR-2 and PAR-3 receptors on eosinophils and neutrophils may be involved in differences in the pathophysiology and recurrence rates of CRS. Recent evidence suggests that both neutrophilic and eosinophilic inflammation persist in the airways of patients with severe asthma. The mechanisms of interaction between neutrophils and eosinophils remain to be elucidated. As eosinophils express PAR-2, neutrophil-derived serine proteases may activate eosinophils. Neutrophil proteases significantly induced superoxide production by eosinophils. Elastase was the most potent among them, while sivelestat and PMSF inhibited the reaction. The proteases induced production of IL-6, IL-8, TNF-α and GRO-α, which may be involved in neutrophilic inflammation [34]. It is known that PAR-2 expression is upregulated in the airway epithelium in asthma [21], but this paper is the first to demonstrate the novel finding of upregulation of PAR-2 and PAR-3 expression on the nasal sinus mucosa in asthma-complicated CRS. Our findings suggest that differences in the expression of PAR-2 and PAR-3 on epithelial cells, eosinophils and neutrophils are involved in the pathogenesis of CRS. Our results also suggest that targeting of PAR-2 and PAR-3 may represent a novel therapeutic approach for CRS.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the contents of this article.

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Expression of IL-33 and Its Receptor ST2 in Chronic Rhinosinusitis With Nasal Polyps

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Objectives/Hypothesis: Interleukin (IL)−33 is a novel member of the IL-1 cytokine family and a ligand for the orphan IL-1 family receptor ST2. IL-33 induces T helper 2-type inflammatory responses and is considered to play a crucial role in allergic inflammatory reactions such as asthma and atopic dermatitis. However, the role of IL-33 and its receptor ST2 in chronic rhinosinusitis remains unclear.

Study Design: In vitro study.

Methods: The expression patterns of IL-33 and ST2 at both mRNA and protein levels in nasal polyps from eosinophilic chronic rhinosinusitis (ECRS) patients (n = 10) and non-ECRS patients (n = 13), as well as in seemingly normal mucosa of the uncinate processes in patients without sinusitis (control; n = 5), were compared using immunohistochemical staining, enzyme-linked immunosorbent assay, and real-time polymerase chain reactions.

Results: ST2-positive cells in the inflammatory cells in the subepithelial layer were significantly higher in the ECRS group than other groups. The expression of ST2 mRNA in polyps of the ECRS group was significantly increased compared with controls. Many ST2-positive eosinophils were observed in the mucosa of ECRS but not in the mucosa of non-ECRS patients. The expression level of IL-33 mRNA was not significantly different among the three groups.

Conclusions: The current study suggests that IL-33 and its receptor ST2 may play important roles in the pathogenesis of chronic rhinosinusitis, especially in ECRS, through the increased expression of ST2 in eosinophils.

Key Words: Cytokine, expression, nasal polyp, eosinophil, rhinosinusitis, IL-33, ST2, inflammatory cells.

Level of Evidence: N/A.

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INTRODUCTION

Chronic rhinosinusitis with nasal polyps (CRSwNP) is an inflammatory disease that remains difficult to treat despite advances in medical and surgical therapy. Recent studies have shown that the majority of patients with CRSwNP in the United States and Europe have pronounced infiltration of eosinophils and expression of interleukin-5 (IL-5) in the nasal polyps.[1] In contrast, more heterogeneity in CRSwNP has been reported in East Asian countries such as Japan, Korea, and China. For example, more than half of CRSwNP cases in Japan do not exhibit eosinophil-dominant inflammation.[2,3] In Japan, CRSwNP is categorized into two subtypes: eosinophilic chronic rhinosinusitis (ECRS), which is similar to the CRSwNP in Western countries; and noneosinophilic chronic rhinosinusitis (non-ECRS), which is characterized by Th1-dominant inflammation.[2] Although a number of hypotheses have been proposed regarding the pathogenesis of CRSwNP,[4,5] the precise molecular mechanisms underlying the development of CRSwNP are still largely unclear.

IL-33 is a recently described cytokine that has been identified as a ligand for the orphan IL-1 family receptor ST2.[6] IL-33 is produced by airway epithelial cells, fibroblasts, and smooth muscle cells. ST2 is expressed in monocytes, mast cells, eosinophils, Th2 lymphocytes,[7] and innate lymphoid cells.[8–10] IL-33 drives the production of Th2 cytokines such as IL-4, IL-5, and IL-13 by Th2 cells, mast cells, basophils, eosinophils, NKT cells, NK cells,[6,11–13] and innate lymphoid cells.[8–10] Recent studies have shown that IL-33 may play an important role in Th2-mediated eosinophilic inflammation,[14] and that polymorphisms within the IL-33 receptor gene are associated with the severity of asthma.[15] In an

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experimental mouse model of allergic rhinitis, IL-33 is promptly released from nasal epithelial cells in response to exposure to the allergen, and is essential for sneezing and the accumulation of eosinophils and basophils in the nasal mucosa by increasing histamine release from the mast cells and inducing production of chemoattractants from the basophils.[16] IL-33 expression is also reported to be increased in cultures of sinonasal epithelial cells in recalcitrant CRSwNP, and it is further enhanced by a bacteria-associated molecular pattern.[17] Very recently, Shaw et al.[18] reported that the expression of ST2 was elevated in inflamed ethmoid sinus mucosa from patients with CRSwNP compared with CRS without nasal polyps (CRSsNP); and controls and innate lymphoid cells within diseased mucosa in CRSwNP produce IL-13 in response to stimulation with recombinant IL-2 and IL-33 within diseased mucosa in CRSwNP.[18] However, the information regarding the in vivo expression of IL-33 and ST2 in the nasal polyps and the normal, non-CRS mucosa is still limited.

Therefore, the aim of the present study was to examine the expression and localization of IL-33 and ST2 in sinonasal polyps in ECRS and non-ECRS patients using immunohistochemical staining, enzyme-linked immunosorbent assay (ELISA), and real time-polymerase chain reaction (RT-PCR). We also examined the identity of ST2 positive cells in the polyps using double-immunostaining for ST2 and cell type-specific molecular markers.

MATERIALS AND METHODS

Patients

CRSwNP was diagnosed based on the criteria of the EAACI position article,[19] in which this disease entity was defined as having two or more of the following symptoms: blockage/congestion, discharge, anterior/posterior drip, facial pain/pressure, reduction or loss of smell for at least 3 months, and endoscopic signs of nasal polyp(s). Patients with CRSwNP associated with chronic obstructive pulmonary disease, diffuse panbronchiolitis, Churg-Strauss syndrome, congenital mucociliary diseases, or cystic fibrosis were excluded from this study. None of the patients included had been treated with systemic corticosteroids or other immune-modulating drugs for at least 1 month prior to surgery, although some patients had received antihistaminic agents and/or macrolide antibiotics.

The patients were classified into two groups: the ECRS group, which was defined as having the eosinophil count of more than 50 per microscopic field (×400 magnification) using five fields located in the subepithelial area of the polyps,[2] and the non-ECRS group, which did not fulfill this criteria. The normal-appearing mucosa of the uncinate processes, which were surgically removed in six patients without CRS (two with frontal sinus cysts, four with maxillary sinus tumors) served as controls. The study was approved by the local ethical committee of The University of Tokyo Hospital (#2656). Informed consent was obtained from each patient before collecting the samples.

Sampling of Tissue Specimens and Histological Procedures

The nasal polyps and control mucosae were harvested during endoscopic sinus surgery. A part of each sample was fixed in 10% formalin, embedded in paraffin, sectioned at 4 μm-thick, mounted on MAS-coated slides (Matsunami Glass, Osaka Japan), and used for hematoxylin-eosin staining as well as the following immunohistochemistry. Another part was immediately immersed in RNA later for RT-PCR Analysis. The rest was immediately frozen and kept at −80°C until use for ELISA.

Immunohistochemistry

The following primary antibodies were used for evaluation of the expression of IL-33 and ST2, as well as the identification of inflammatory cells in the specimens: anti-IL-33 (mouse monoclonal, clone Nessy-1; Alexis Biochemicals, CA); anti-ST2 (mouse monoclonal, clone HB12;Medical & Biological Laboratories, Nagoya, Japan); anti-eosinophil major basic protein (MBP) (mouse monoclonal, clone BMK-13; Millipore, CA), anti-mast cell tryptase (mouse monoclonal, clone AA1, Thermo Fisher Scientific; CA), anti-human plasma cells (mouse monoclonal, clone VS38c; Dako Cytomation Japan, Kyoto, Japan) and anti-CD3 (rabbit monoclonal, clone SP7; Nichirei, Tokyo, Japan).

For single immunostaining for IL-33, ST2, MBP, mast cell tryptase, plasma cells, and CD3, immunoreactivity was made visible by diaminobenzidine (DAB) (Simplestain DAB, ready-to-use; Nichirei). To ensure that there was no nonspecific staining of secondary antibodies, the primary antibodies were omitted from the reaction.

For double-immunostaining for MBP and ST2, we chose enzymatic visualization of the immunoreactivity because the reliable primary antibodies for MBP and ST2 were both raised in mouse; thus, double immunofluorescence staining was impossible. MBP immunoreactivity was made visible by the DAB reaction (Simplestain DAB, Nichirei). After MBP staining by DAB, the sections were placed in citrate buffer solution (Dako Cytomation, Japan) and autoclaved at 121°C for 20 minutes to abolish the antigenicity of the anti-MBP antibody to the secondary antibody[19] and to retrieve ST2 antigenicity. The sections were then incubated with mouse anti-ST2 antibody, and immunoreactivity was made visible by the Vector Red kit (Vector Labs, Burlingame, CA).

Double-immunostaining for mast cell tryptase-ST2 and CD3-ST2 were also performed, using the enzymatic visualization described above for MBP and ST2 double immunostaining.

The details of immunohistochemical procedures are provided in Supplementary file 1.

ELISA for IL-33 and ST2

The nasal mucosae were homogenized with 10 times as much volume of CelLytic MT Cell Lysis Reagent (Sigma-Aldrich, Tokyo, Japan), and with a protease inhibitor cocktail (P8340 Sigma-Aldrich, Tokyo, Japan) and benzonase endonuclease (E1014 Sigma-Aldrich, Tokyo, Japan). Homogenized samples were centrifuged at 4°C at 15,000 g for 10 minutes. IL-33 and ST2 protein concentrations in the supernatants were determined by an enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Tokyo, Japan) according to the manufacturer's instructions. Absorbance was read at 450 nm on a microplate reader.

Real-Time Quantitative PCR Analysis

The sample tissues were lysed in ISOGEN (Nippon Gene, Tokyo, Japan), and the total RNA was extracted according to the manufacturer's instructions. The mRNA expression was analyzed using an Applied Biosystems 7500 Real Time PCR System (PE Applied Biosystems, Foster City, CA). The primers and the probes for human β-actin, IL-33, and ST2 were

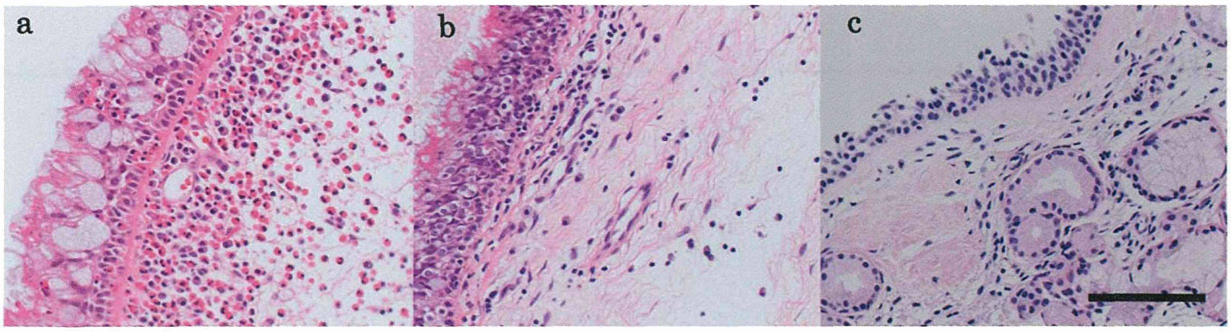

Fig. 1. Representative H-E-stained histological sections of nasal polyps obtained from ECRS (a), non-ECRS (b), and non-CRS (c) groups. In the ECRS group, almost all of the infiltrating cells are eosinophils, whereas most of the infiltrating cells are lymphocytes in the non-ECRS group and few inflammatory cells are infiltrating in the non-CRS group. Scale bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

designed by PE Applied Biosystems. For each sample, the differences in threshold cycles between the cytokine and β-actin genes (ΔCt sample, ΔCt control) were determined, a calibrated ΔCt value (ΔΔCt, ΔCt sample − ΔCt control) was calculated, and then the relative quantitation (RQ) values were calculated using the following equation: $RQ = 2^{-\Delta\Delta Ct}$.

Cell Counting

To determine the degree of eosinophil infiltration in the tissues, two of the authors (S.B., KENJI K.) independently counted the number of infiltrated cells in five random fields using H-E sections manually under light microscopy at high magnification (×400) in a blinded manner. The number of mast cells, T lymphocytes, and ST2-positive cells was counted in a same manner using sections immunostained for mast cell tryptase, CD3 and ST2, respectively.

Statistical Analyses

Statistical analyses were done using SPSS statistical software (SPSS, Chicago, IL). All data are expressed as mean ± standard error in each group. The significance of the differences in cell number and mRNA expression between groups was determined using the Mann-Whitney U test. The significance of the differences in protein concentrations by ELISA between groups was determined using a *t* test. A difference was considered significant if $P < .05$.

RESULTS

The ECRS group included 10 patients (no females and 10 males, age range 31–73 years, mean age 54.3 years), in which the average eosinophil count in the total white cell count in peripheral blood was 9.0% (range 5.0%–23.0%) and the average of the number of eosinophils was 587.8/mm^3 (range 308-1817/mm^3). Six patients in this group had allergic rhinitis, three had asthma, and one had aspirin sensitivity, while three reported no additional complications. The non-ECRS group included 13 patients (4 females and 9 males, age range 40–72 years, mean age 55.8 years), in which the average eosinophil count in the total white cell count in peripheral blood was 2.1% (range 0.4-4.5%) and the average number of eosinophils was 140.0/mm^3 (40-329.4/mm^3). Twelve of these patients did not have any complications; one patient had allergic rhinitis. No patients in the non-ECRS group had asthma or aspirin sensitivity. There was no significant difference in age between the ECRS and non-ECRS groups, whereas the peripheral blood eosinophil count was significantly greater ($P < .001$) in the ECRS group compared with the non-ECRS group.

Histological observations of the nasal polyps showed that, as expected, eosinophils were the predominant type of infiltrating cells in the ECRS group (Fig. 1a). On the other hand, most of the infiltrating cells were lymphocytes and the plasma cells in the non-ECRS group (Fig. 1b) and few inflammatory cells are infiltrating in the non-CRS group (Fig. 1c).

Immunohistochemical Analysis of Inflammatory Cells in Nasal Tissues

We counted the number of cells positive for MBP (eosinophils), mast cell tryptase (mast cells), VS38c (plasma cells), and CD3 (T-cells) using immunohistochemical staining in ECRS polyps, non-ECRS polyps, and control mucosa. Typical immunohistochemical pictures are shown in Figure 2. As illustrated in Figure 3, the median (interquartile range [IQR]) counts for eosinophils were significantly higher in ECRS polyps (145.2; 53.2–368.8) compared with control mucosa (0; 0–3.8; $P < .0001$) and non-ECRS polyps (3.4; 0–47.8; $P < .0001$). The median counts for plasma cells were significantly higher in non-ECRS polyps (34.8; 16.8–84.2) compared with control mucosa (6.1; 2.0–7.4; $P < .0001$) and ECRS polyps (20.9; 2.4–30.8; $P < .001$). No significant differences were observed in the median counts for mast cells or T-cells among the groups ($P > .05$).

Immunohistochemical Localization of ST2 and IL-33

Representative microphotographs of ECRS polyps immunostained for ST2 are shown in Figure 4. ST2 immunoreactivity was localized in epithelial cells, capillary endothelial cells, and glandular cells in all three groups (Fig. 4a, b), and was also pronounced in inflammatory cells in the subepithelial layer of the ECRS group (Fig. 4c). The median counts for ST2-positive

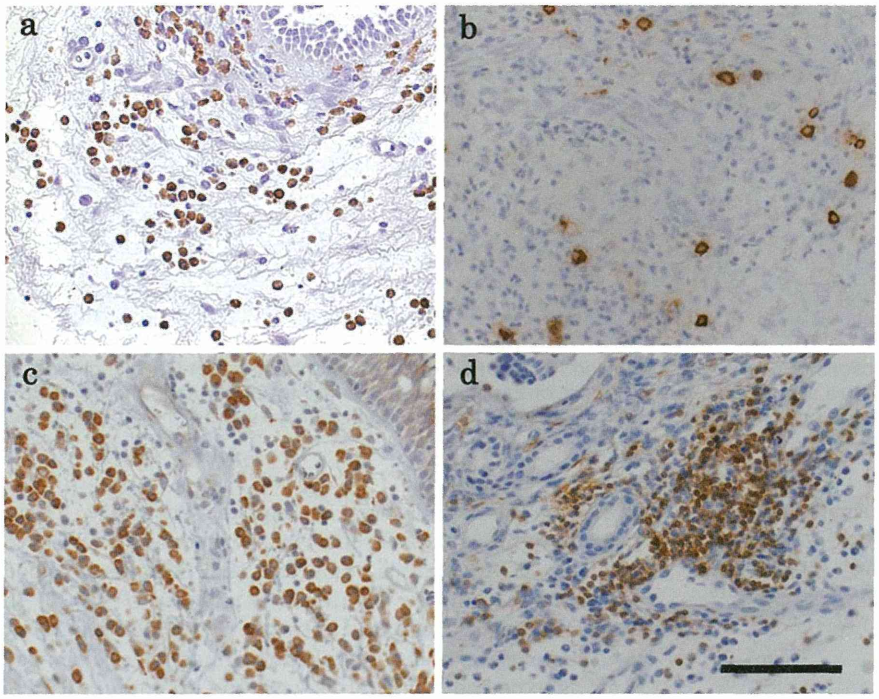

Fig. 2. Photographs showing immunohistochemical staining for MBP (eosinophils) (a), mast cell tryptase (mast cells) (b), VS38c (plasma cells) (c), and CD3 (T-cells) (d). Scale bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cells in the subepithelial layer were significantly higher in the ECRS group (n = 10, 46.2; 18.8–225.6) compared with the control (n = 6, 1.5; 0–5.2; $P < .0001$) and non-ECRS groups (n = 13, 10.2; 0.4–34; $P < .0001$) (Fig. 3e). IL-33 was expressed in the nuclei of epithelial cells and capillary endothelial cells in all groups, and the staining pattern was similar among groups (Fig. 4d–4f).

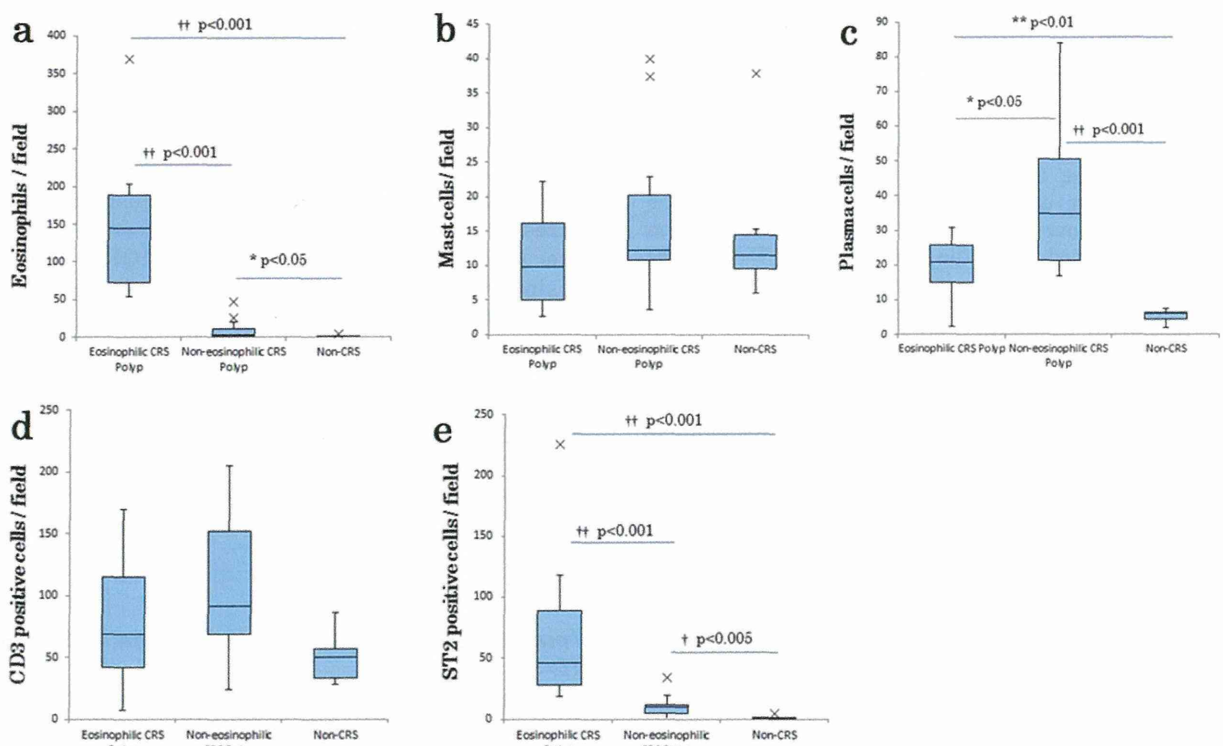

Fig. 3. The number of eosinophils (H-E) (a), mast cells (b), plasma cells (c), T cells (d), and ST2-positive cells in the subepithelial layer (e) per mm^2 in the polyps of ECRS and non-ECRS cases, as well as in the mucosa of non-CRS controls. Data in box-and-whisker plots represent the median, lower, and upper quartile and the minimum to maximum value. × = outliers (††$P < 0.001$, **$P < 0.01$, *$P < 0.05$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

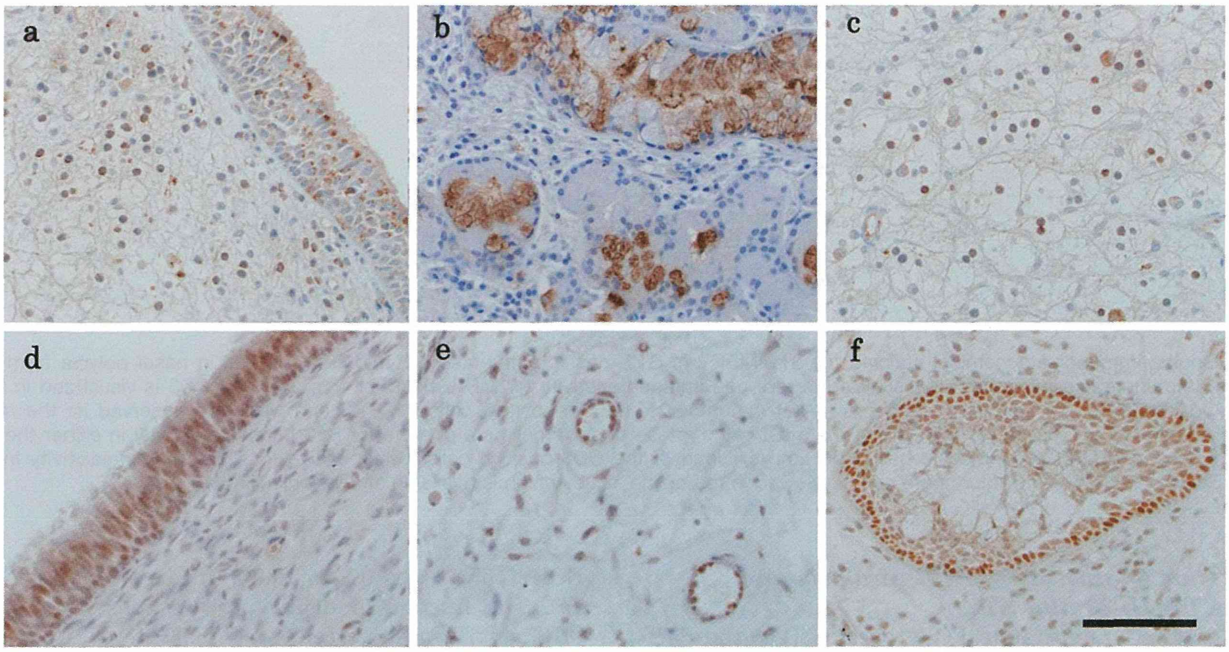

Fig. 4. Immunohistochemistry for ST2 (a–c) and IL-33 (d–f) in nasal polyps from ECRS and non-ECRS patients. (a–c) Expression of ST2 is observed in the nasal epithelium, capillary endothelial cells (a), and glandular cells (b)—and also in inflammatory cells (c) in the subepithelial layer of ECRS and non-ECRS polyps. Scale bar = 100 μm. (d–f) IL-33-immunoreactivity is observed in the nuclei of nasal epithelium (d), capillary endothelial cells (e), and glandular cells (f) from patients with ECRS and non-ECRS. Scale bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

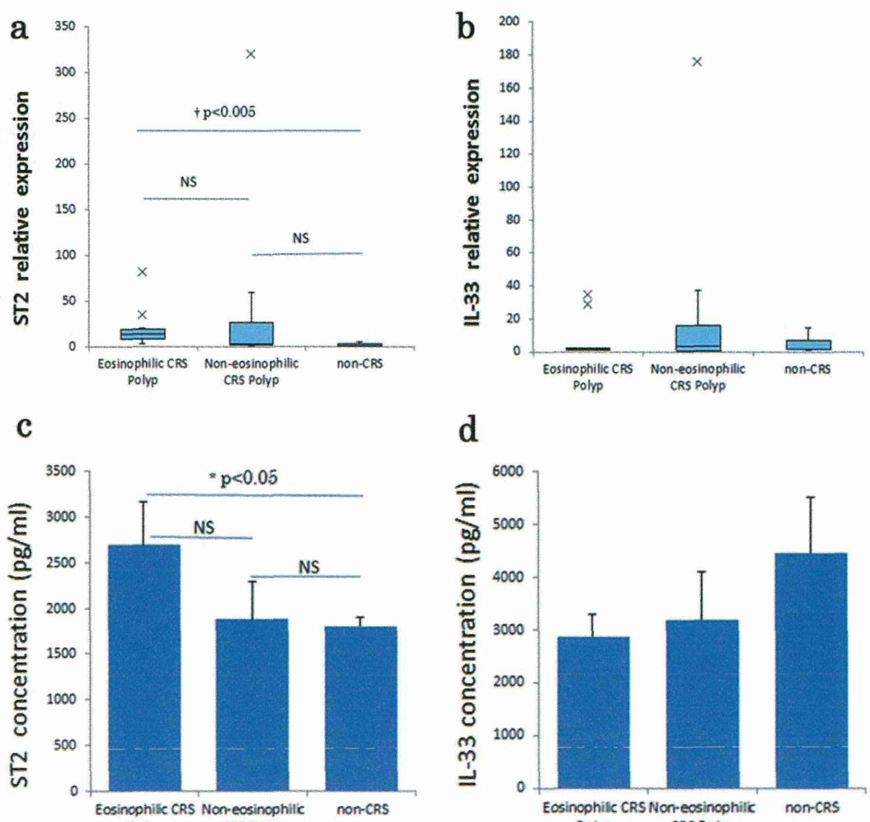

Fig. 5. (a,b) The relative levels of ST2 (a) and IL-33 (b) mRNA in nasal tissue determined by real-time PCR. Box-plot analysis of gene expression profiling dataset of ECRS polyps (n = 10), non-ECRS polyps (n = 13), and control mucosa (n = 5) from two independent experiments. The box represents the distribution of values; a line across the box represents the median; the box stretches from the lower hinge (the 25th percentile) to the upper hinge (the 75th percentile). × = outliers (†$P < 0.005$). (c,d) Total ST2 (c) and IL-33 (d) protein concentrations in the polyps of ECRS (n = 8), non-ECRS cases (n = 6), and in the mucosa of non-CRS controls (n = 5) determined by ELISA. Error bars represent mean ± SE, *$P < 0.05$ vs. controls. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

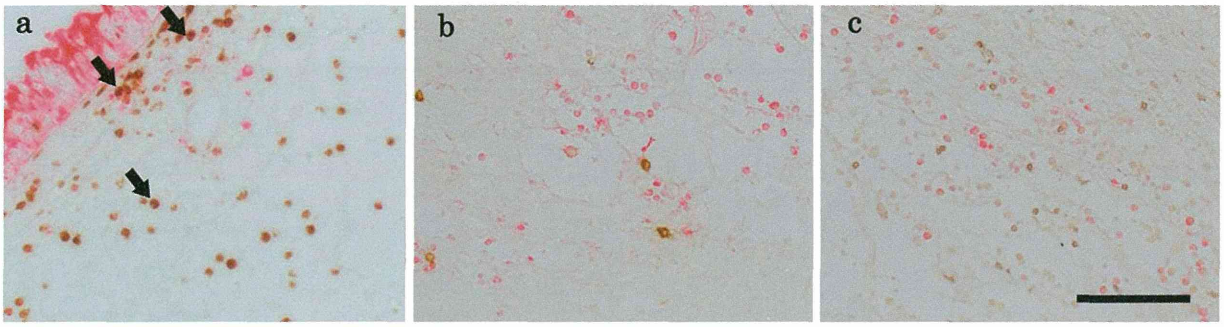

Fig. 6. Double-immunohistochemical staining for ST2-MBP (a), ST2-mast cell tryptase (b), and ST2-CD3 (c) in nasal polyps from ECRS patients. Immunoreactivity for ST-2 is visualized in red, and immunoreactivity for MBP, mast cell tryptase, and CD3 is visualized in brown. Double-positive cells are colored in dark brown. (a) A number of double-positive cells for ST-2 and MBP are observed in the mucosa (arrows). Scale bar = 100 μm. (b) Very few of the mast cell tryptase positive cells are positive for ST2 immunoreactivity in either the ECRS group or the non-ECRS group. Scale bar = 100 μm. (c) None of the CD3-positive cells were positive for ST2 immunoreactivity in either group. Scale bar = 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Real-Time Quantitative PCR Analysis and ELISA for IL-33 and ST2

Real-time quantitative PCR revealed that the ST2 mRNA expression level was significantly higher in ECRS polyps compared with control mucosa ($P < .0005$) but not significantly different between non- ECRS polyps and control mucosa (Fig. 5a). The expression of IL-33 mRNA was not significantly different among the groups (Fig. 5b). The concentration of ST2 protein in supernatants prepared from tissue homogenates examined by ELISA was significantly higher in the ECRS polyps compared with the control nasal mucosa ($P < .05$), but it was not significantly different between non-ECRS polyps and control mucosa (Fig. 5c). The concentration of IL-33 protein was not significantly different among the groups (Fig. 5d).

Identity of ST2-Positive Cells

The identity of ST2-positive cells in the subepithelial layer was examined using double immunohistochemistry (Fig. 6). The fraction of double-positive cells for ST2 and MBP in MBP-positive eosinophils ranged from 14.9% to 58.7% (median 38.3%, n = 7) in ECRS polyps, whereas the fraction of double-positive cells in non-ECRS polyps was 0% to 10.3% (median 0%, n = 7) (Fig. 7a). The fraction of double-positive cells for ST2 and MBP in ST2-positive cells was 78.9% to 97.1% (median 89.8%, n = 7) in ECRS polyps, whereas the fraction of double-positive cells in non-ECRS polyps was 0% to 15.5% (median 0% n = 7) (Fig. 7b). The fraction of double-positive cells in MBP-positive cells and ST2-positive cells was significantly higher in the ECRS group compared with the non-ECRS group ($P < .0005$). There were very few double-positive cells for mast cell tryptase and ST2 in either ECRS polyps or non-ECRS polyps. Virtually no double-positive cells for CD3 and ST2 were observed in either group (data not shown).

DISCUSSION

The present study demonstrated that the concentration of IL-33 protein and the expression level of IL-33 mRNA in both ECRS and non-ECRS polyps were not significantly different from that in the control mucosa. Shaw et al.[18] have reported a similar result regarding CRS, that no significant difference in the relative expression of IL-33 mRNA was observed among the inflamed ethmoid sinus mucosa from patients with CRSwNP than from patients with CRSsNP and control

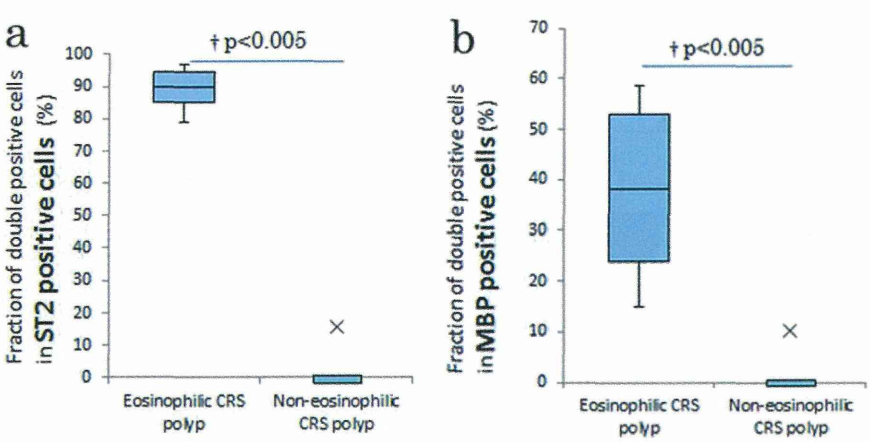

Fig. 7. The fraction of double positive cells for MBP and ST2 in MBP- and ST2-positive cells, respectively. In the non-ECRS group, there were no ST2-positive eosinophils except for one case. In contrast, ST2-positive eosinophils were observed in all of the ECRS cases. (a) The fraction of double positive cells in MBP-positive cells (%). (b) The fraction of double positive cells in ST2-positive cells (%) Data in box-and-whisker plots represent the median, lower, and upper quartile, and the minimum to maximum value. × = outliers ($^{\dagger}P < 0.005$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mucosa. These contrast with the results of recent studies showing that the expression of IL-33 is increased in the respiratory mucosa of the Th2-type allergic inflammatory diseases, such as allergic rhinitis[16,20] and asthma.[15,21,22] Our results suggest that the amount of IL-33 production may not be increased in the CRS polyps, at least in the steady-state condition.

On the other hand, the protein concentration and mRNA expression of ST2 was significantly greater in ECRS polyps in comparison with the control mucosa. Also, a significantly greater number of ST2-positive eosinophils were observed in the ECRS polyps compared with controls and non-ECRS polyps. In the non-ECRS group, the fraction of ST2-positive eosinophils out of total eosinophils was 0% to 10.3%, while in ECRS polyps this fraction was 78.9% to 97.1%. This suggests that in ECRS, ST2 expression is upregulated in eosinophils. A similar increase in expression of ST2 has been reported in other allergic diseases, such as allergic rhinitis,[20] atopic dermatitis,[23] and CRSwNP. [18] Since the present study did not show colocalization of ST2 and CD3, this would suggest that IL-33 does not act directly on T cells, at least in the sinus mucosa.

A finding that is difficult to interpret is that the upregulation in ST2 expression in ECRS eosinophils is not reflected as a significant increase in ST2 protein expression in ECRS polyps compared with non-ECRS polyps (Fig. 5). It may be due to the ubiquitous expression of ST2 in polyp glandular cells (Fig. 4b). This may have masked the significant difference in ST2 protein expression in eosinophils.

Eosinophils have been identified as one of the target cells of IL-33 signaling,[24–26] but the mode of ST2 expression by eosinophils remains under debate. For example, Wong et al. showed by means of Western blot analysis that ST2 protein is constitutively expressed by eosinophils.[27] On the other hand, Cherry et al.[14] reported that freshly isolated eosinophils in the peripheral blood do not express ST2 protein, although they constitutively express ST2 mRNA. Eosinophils express ST2 protein on their surface after being cultured, and this expression increases upon incubation with granulocyte macrophage colony-stimulating factor.[14] This discrepancy between studies may suggest that the amount of ST2 protein expression by eosinophils changes considerably depending on the surrounding environment; and as yet unspecified signals in ECRS patients may increase the expression of ST2 by eosinophils. The discrepancy may also be due to differences in the sensitivity of the detection system used in each study. In our study, it is possible that the eosionphils in non-ECRS polyps express ST2 but at a very low level that was not detected by our immunohistochemical methodology.

IL-33 enhances adhesion and survival of eosinophils,[25] as well as the production of proinflammatory cytokines by eosinophils.[24] It has also been reported that IL-33 and ST2 on eosinophils are important for trafficking eosinophils in the allergic lung.[28] The eosinophils could be one of the targets of IL-33 in the inflammatory cell population of nasal polyps in ECRS patients. A recent report by Shaw et al.[18] also suggests that innate lymphoid cells and IL-33 may play a key role in the pathophysiology of CRSwNP. They demonstrated that innate lymphoid cells in the inflamed sinonasal mucosa produce IL-13 in response to stimulation with recombinant IL-2 and IL-33,[18] which could promote mucous production and tissue eosinophilia.

In the present study, we compared polyps in CRS patients with uncinate mucosa from non-CRS patients. In this study, we were unable to examine tissues from different sinonasal regions within the same group, or tissues from the same sinonasal regions in different groups, due to the availability of such tissue samples. We intend to address these issues in the future.

Another limitation of our study is that it did not test the possibility that the release of IL-33 is changed in CRS polyps. An experimental mouse model of allergic rhinitis[16] and a human study regarding allergic rhinitis[29] have demonstrated that the release of IL-33 from the mucosa increases in response to stimulation by allergens. It remains unclear whether IL-33 release is increased or not in CRS polyps. Further study is necessary to address this issue by examining IL-33 levels in the nasal lavage of the CRS patients.

CONCLUSION

The present study demonstrates that the number of ST2-positive inflammatory cells in the subepithelial layer is significantly higher in the ECRS group than other groups. Double-immunostaining showed that the majority of such ST2-positive cells were eosinophils. The expression of ST2 mRNA and the concentration of ST2 protein in polyps of the ECRS group were also significantly increased compared with controls. In contrast, the expression level of IL-33 mRNA and the concentration of IL-33 were not different among the groups. These findings suggest that IL-33 and its receptor ST2 may play important roles in the pathogenesis of chronic rhinosinusitis, especially in ECRS, through the increased expression of ST2 in eosinophils.

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JOHNS
特集●図でみる免疫学のABC●

免疫と耳鼻咽喉科関連疾患の病態

好酸球性副鼻腔炎

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Shintaro BABA Kenji KONDO

● Key Words ●好酸球性副鼻腔炎，サイトカイン●

はじめに

好酸球性副鼻腔炎は鼻茸の再発を高率に認める難治性の副鼻腔炎である。その病態生理は依然不明な点が多い。本稿では，各炎症細胞の細胞動態の組織学的解析および組織中のサイトカインの発現解析を通じて好酸球性副鼻腔炎の病態生理について文献的知見，当科で得られた知見を解説する。

Ⅰ．臨床像

典型例は成人発症の副鼻腔炎で，両側性かつ多発性の浮腫性鼻茸を示す（図1）[1]。好酸球性副鼻腔炎の所見は組織学的に鼻茸や副鼻腔粘膜に好酸球優位な炎症細胞浸潤がみられることである。また，上皮細胞の剥脱や分泌細胞の増加，基底膜の肥厚もみられ，病理学的には喘息に酷似する（図2）。ニカワ状の粘稠な分泌物の貯留を認め，粘液内にも好酸球滲出がみられる（好酸球性ムチン）。中鼻甲介周囲（中鼻道，嗅裂）の病変が強いため，早期より嗅覚障害を訴える。上顎洞に比べ篩骨洞病変が優位である（図3）が，進行すると汎副鼻腔病変となる。

また，鼻茸のサイズが大きくなると鼻閉を訴える。喘息を合併することが多く，特にアスピリン喘息を合併する場合は難治である。アレルギー性鼻炎の関与は少なく，IgE値はさまざまである[2]。一方，血中好酸球増多がみられることが多い。マクロライド療法の効果は限定的ではあるが，ステロイド薬の全身投与にはよく反応する。

東京大学耳鼻咽喉科鼻外来を2002年から2009年に受診した好酸球性副鼻腔炎症例136例について臨床像を検討したところ，性別は男性83例（61％），女性53例（39％）と男性の方が多い傾向にあった。男性の方が20歳以下での発症数が多く，平均年齢は男性の方が有意に若かった（平均男性42.2歳，女性48.0歳，$p<0.05$，T test）。血中好酸球値（％）は平均で男性10.6％，女性12.0％と女性の方が高い傾向であるが，統計学的有意差

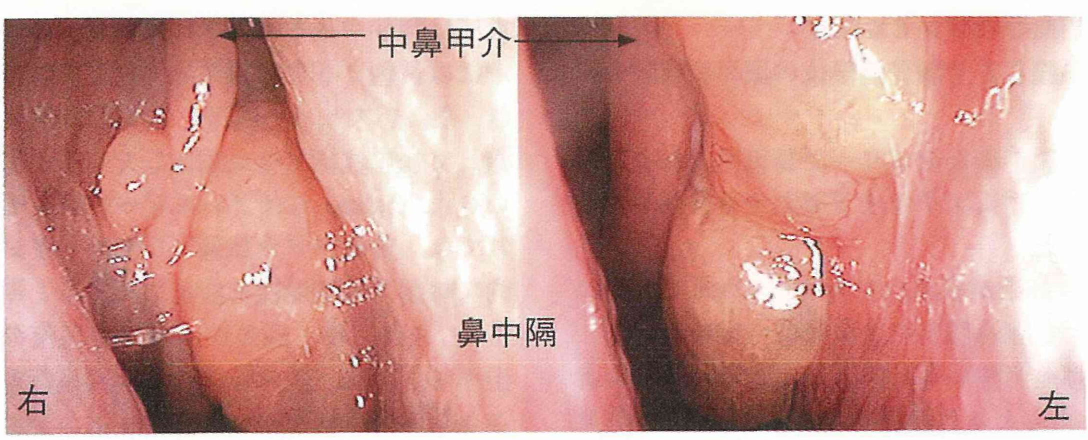

図1　好酸球性副鼻腔炎症例の鼻内所見（嗅裂，中鼻道に多発性ポリープを認める）

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