



Distribution, subtype population, and IgE positivity of mast cells in chronic rhinosinusitis with nasal polyps

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ARTICLE INFO

Article history:

Received for publication March 16, 2017.

Received in revised form May 17, 2017.

Accepted for publication May 18, 2017.

ABSTRACT

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) has been categorized into 2 subtypes in the Asian population: eosinophilic chronic rhinosinusitis (ECRS; similar to CRSwNP in Western countries) and non-ECRS (characterized by inflammation dominated by T-helper cell type 1). The pathogenesis of CRSwNP and the role of mast cells are poorly understood.

Objective: To investigate the distribution, phenotypes, and immunoglobulin E (IgE) positivity of mast cells in these 2 subtypes of CRSwNP.

Methods: We collected nasal tissue from patients with CRSwNP and control subjects. The mRNA for mast cell proteases tryptase and chymase was measured using real-time polymerase chain reaction, and the distribution of each type of protease-positive mast cell was examined using immunohistochemistry and immunofluorescence. IgE distribution on mast cells was determined using double-immunofluorescent staining for IgE and tryptase.

Results: Expression of tryptase mRNA was significantly increased in nasal polyps from patients with the 2 subtypes of CRSwNP compared with controls. Immunohistochemistry showed increased numbers of tryptase-positive mast cells in the epithelium, glands, and submucosa of ECRS polyps, whereas the number of tryptase- and chymase-positive mast cells was increased in the glands and submucosa of non-ECRS polyps. IgE-positive mast cells were abundant in the epithelial, glandular, and submucosal regions of ECRS polyps but few were detected in non-ECRS polyps.

Conclusion: The present study demonstrates that the distribution, subtype population, and IgE positivity of mast cells is different between ECRS and non-ECRS nasal polyps. Our results suggest a role for IgE-mediated mast cell activation in the pathogenesis of ECRS.

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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. Most patients with CRSwNP in the United States and Europe have pronounced infiltration of eosinophils and expression of interleukin (IL)-5 in nasal polyps.¹ In

contrast, more heterogeneity in CRSwNP has been reported in East Asian countries, such as Japan and China. For example, more than half the CRSwNP cases in Japan do not exhibit eosinophil-dominant inflammation.^{2,3} In Japan, CRSwNP is categorized into 2 subtypes: eosinophilic chronic rhinosinusitis (ECRS), which is similar to the CRSwNP in Western countries, and non-ECRS, which is characterized by inflammation dominated by T-helper cell type 1.²

We previously found higher immunoglobulin (Ig) E protein concentration and significant upregulation of IL-5 mRNA in ECRS polyps compared with non-ECRS polyps and non-CRS control specimens in the Japanese population.⁴ Also, mRNA expressions of IL-13 and ϵ germline gene transcripts were significantly higher in ECRS polyps compared with non-ECRS polyps and non-CRS control specimens. Furthermore, we showed that the number of IgE-positive cells in ECRS polyps was significantly larger compared with non-ECRS polyps and non-CRS control specimens and that

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Disclosures: Authors have nothing to disclose.

Funding Sources: Grants-in-Aid for Young Scientists (B), Health Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan; Environmental Restoration and Conservation Agency of Japan; Japan Society for the Promotion of Science; Practical Research Project for Rare/Intractable Diseases from Japan Agency for Medical Research and Development; and AMED.

the localization of IgE in inflammatory cells was predominantly confined to mast cells in ECRS polyps.⁴ Together these data suggest that, in nasal polyps in ECRS, B cells undergo a class-switch recombination from IgM and IgG to IgE in situ, that an ongoing, increased synthesis of mature IgE occurs, and that IgE formed in the polyp might contribute to the development of chronic inflammation by IgE-combined mast cell degranulation in patients with ECRS. This hypothesis appears to be supported by the therapeutic effect of anti-IgE monoclonal antibody (omalizumab) on CRSwNP.^{5,6}

Mast cells produce an abundance of cytokines that activate eosinophils, directly promote tissue remodeling, and produce profound tissue edema.⁷ In human mast cells, 2 subtypes have been recognized by their expression of granular neutral proteases: mast cell positive for tryptase (MC-T) and mast cell positive for tryptase and chymase (MC-TC). The MC-T type predominantly expresses high levels of tryptase but not chymase or carboxypeptidase A3, whereas the MC-TC type expresses all 3 proteases.⁸

Shaw et al⁹ found an increased percentage of mast cells in CRSwNP using flow cytometry. Abonia et al¹⁰ and Hsu Blatman et al¹¹ reported that intraepithelial mast cells in these patients showed a MC-T but not a MC-TC phenotype. Takabayashi et al¹² reported that mast cells were significantly increased in mucosal and glandular epithelium but not within the lamina propria in nasal polyps from patients with CRSwNP in a study conducted in patients in the United States. They also found that mucosal epithelial mast cells showed MC-T phenotypes, whereas glandular epithelial mast cells showed MC-TC phenotypes.¹² Cao et al¹³ also reported a significant increase of MC-T in the mucosal epithelium of eosinophilic nasal polyps in China. These results suggest that mast cells could be involved in the pathogenesis of CRS.

In this study, we examined the distribution and phenotype of mast cells in ECRS and non-ECRS nasal polyps. We also evaluated the patterns of IgE distribution on mast cells in ECRS and non-ECRS polyps.

Methods

Patients

The diagnosis of CRSwNP was based on the American Academy of Otolaryngology–Head and Neck Surgery clinical practice guideline,¹⁴ which defines CRSwNP as having at least 2 of the following symptoms: blockage or congestion, discharge, anterior or posterior drip, facial pain or pressure, decrease 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 before surgery, although 12 of 17 patients with ECRS and 11 of 17 patients with non-ECRS had received antihistaminic agents and 5 of 17 patients with ECRS and 9 of 17 patients with non-ECRS had received macrolide antibiotics.

Patients were classified into 2 groups: the ECRS group was defined as having an eosinophil count of more than 70 per microscopic field ($\times 400$ magnification) using 5 fields located in the subepithelial area of the polyps¹⁵ and the non-ECRS group did not fulfill this criterion. The normal-appearing mucosa of the uncinate processes, which were surgically removed in 7 patients without CRS (3 with frontal sinus cysts and 4 with maxillary sinus benign tumors without inflammation in the uncinate processes) served as the non-CRS control group.

Before surgery, each patient underwent computed tomography of the sinuses, and the Lund-Mackay computed tomographic score was determined.¹⁶ This score was calculated by assigning opacification scores of 0, 1, or 2 for the maxillary, anterior ethmoid,

posterior ethmoid, frontal, and sphenoid sinuses and scores 0 or 2 for the ostiomeatal complex for the right and left sides. The total score ranged from 0 to 24.

The study was approved by the ethical committee of The University of Tokyo Hospital (#2656; Tokyo, Japan). Written informed consent was obtained from each patient before collecting samples.

Sampling of Tissue Specimens and Histologic 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 μ m thick, mounted on MAS-coated slides (Matsunami Glass, Osaka, Japan), and used for hematoxylin and eosin staining and for immunohistochemistry. The rest of the tissue sample was immediately immersed in RNA Later (Life Technologies, Carlsbad, California) and used for real-time polymerase chain reaction (PCR) analysis. However, we could not obtain sufficient mucosal tissue in some cases and therefore could not prepare the specimen for some real-time PCR and immunohistochemistry analyses (see Results and Table 1).

Immunohistochemistry

The following primary antibodies were used for evaluation of the localization of IgE, mast cell tryptase and chymase, and the identification of inflammatory cells in the specimens: anti-IgE (rabbit polyclonal antibody against ϵ -heavy chain; Nichirei, Tokyo, Japan), anti-mast cell tryptase (mouse monoclonal antibody against clone AA1; Thermo Fisher Scientific, Waltham, Massachusetts), and anti-mast cell chymase (mouse monoclonal antibody against clone CC1; Thermo Fisher Scientific).

For single immunostaining for IgE, mast cell tryptase, and mast cell chymase, sections rehydrated through a xylene and ethanol series were immersed in 10 mmol/L of citrate buffer solution (pH 6.0; Dako Cytomation Japan, Kyoto, Japan) and autoclaved at 121°C for 20 minutes for the retrieval of antigens. Endogenous peroxidase activity was blocked by treatment with 3% hydrogen peroxide in methanol for 15 minutes at room temperature (RT). Next, sections were incubated for 30 minutes in a blocking solution (phosphate buffered saline [PBS], pH 7.4, containing 2% bovine serum albumin [Sigma-Aldrich Japan, Shinagawa-ku, Japan], 0.1% Triton X-100, and 0.1% sodium azide) at RT to decrease nonspecific antibody binding

Table 1
Patient Profiles

	ECRS	Non-ECRS	Non-CRS
Subjects (M/F), n	17 (17/0)	17 (13/4)	7 (5/2)
Age (y) ^a	58.3 (42–76)	58.6 (42–72)	51.6 (30–69)
Asthma	2/17	0/17	0/7
Allergic rhinitis ^b	8/17	4/17	0/7
Eosinophil count (% of total white cells) ^c	6.5 (5.0–7.2) ^d	1.8 (0.9–3.0) ^d	1.8 (1.5–3.6)
Eosinophil count in nasal tissues ^c	99.2 (70.5–385.2) ^e	3.2 (0–47.8) ^e	0.0 (0–1.0)
Lund-Mackey score ^a	13.8 (5–22) ^d	9.4 (3–14) ^d	—
Methodology used			
Tissue RNA, n (M/F)	11 (11/0)	11 (8/3)	7 (5/2)
Age (y) ^a	66.7 (48–76)	56.2 (42–70)	51.6 (30–68)
IHC, n (M/F)	16 (16/0)	16 (13/3)	7 (5/2)
Age (y) ^a	58.3 (42–76)	58.4 (42–72)	51.6 (30–69)
Double IHC, n (M/F)	10 (10/0)	10 (8/2)	—
Age (y) ^a	57.9 (48–73)	57.8 (43–65)	—

Abbreviations: CRS, chronic rhinosinusitis; ECRS, eosinophilic chronic rhinosinusitis; F, female; IHC, immunohistochemistry; M, male.

^aData are expressed as mean (range).

^bAllergic rhinitis was diagnosed by radioimmunosorbent assay.

^cData are expressed as median (range).

^d $p < .05$.

^e $p < .001$.

and then incubated with anti-IgE antibody (1:750 in blocking solution) overnight at 4°C, anti-mast cell tryptase antibody (1:2,000 in blocking solution) overnight at 4°C, and anti-mast cell chymase antibody (1:2,000 in blocking solution) overnight at 4°C. These antigen retrieval and incubation conditions were determined based on the results of preliminary experiments (data not shown). After several washes in PBS (pH 7.4), sections were incubated for 30 minutes at RT with horseradish peroxidase conjugated with anti-mouse or rabbit IgG antibodies (Simplestain MAX-PO, [M] and [R], ready-to-use; Nichirei, Tokyo, Japan) corresponding to the primary antibodies. After more washes with PBS (pH 7.4), immunoreactivity was made visible with diaminobenzidine (DAB; Simplestain DAB, ready-to-use; Nichirei). After washing with distilled water, the sections were counterstained with hematoxylin, dehydrated, and mounted. To ensure that there was no nonspecific staining of primary antibodies, the primary antibodies were omitted from the reaction.

For double immunostaining for mast cell tryptase and chymase, we chose enzymatic visualization of the immunoreactivity, because the reliable primary antibodies for tryptase and chymase were raised in mouse; hence, double-immunofluorescence staining was impossible. Rehydrated sections were immersed in 10 mmol/L of citrate buffer solution (pH 6.0; Dako Cytomation Japan, Tokyo, Japan) and autoclaved at 121°C for 20 minutes to retrieve mast cell chymase antigenicity. After the blocking procedure described earlier, the sections were incubated with anti-chymase antibody (1:2,000 in blocking solution) at 4°C overnight. After being washed in PBS (pH 7.4), the sections were incubated with horseradish peroxidase-conjugated anti-mouse IgG secondary antibody (Simplestain MAX-PO [M]) for 30 minutes at RT. Chymase immunoreactivity was made visible by the DAB reaction (Simplestain DAB). After more washes in PBS (pH 7.4), the sections were again placed in citrate buffer solution (Dako Cytomation Japan) and autoclaved at 121°C for 20 minutes to abolish the antigenicity of the anti-chymase antibody to the secondary antibody. After several washes in PBS (pH 7.4), the sections were incubated with mouse anti-tryptase antibody (1:2,000 in blocking solution) at 4°C overnight. After several PBS (pH 7.4) washes, the sections were incubated for 10 minutes at RT with a biotinylated anti-mouse secondary antibody (Nichirei). After washes in PBS (pH 7.4), the sections were incubated with avidin-biotin alkaline phosphatase complex solution (SAB-AP Kit; Nichirei) for 10 minutes at RT. After washes in PBS (pH 7.4), immunoreactivity was made visible using the Nichirei Fast Red II kit (Nichirei). After being washed with distilled water, the sections were dehydrated and mounted.

Immunofluorescent Double Staining

Paraffin sections were rehydrated through a xylene and ethanol series before antigen retrieval treatment. Then, the mixture of primary antibodies for IgE and mast cell tryptase was applied overnight at 4°C. The sections were incubated with a mixture of fluorescence-labeled secondary antibodies: a goat anti-rabbit Alexa 488 antibody for IgE and a goat anti-mouse Alexa 594 antibody for mast cell tryptase (all purchased from Molecular Probes, Leiden, The Netherlands). Nuclei were stained using 4',6-diamidino-2-phenylindole (Molecular Probes, Eugene, Oregon) and the slides were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, California).

To confirm the absence of nonspecific staining by secondary antibodies, some sections were incubated with a blocking solution instead of primary antibodies, followed by incubation with the secondary antibodies. The occurrence of potential cross-reactions between antibodies was checked for IgE and mast cell tryptase antibodies by making cross-combinations of primary and secondary antibodies.

Images were acquired with the Radiance 2100 confocal system mounted on a Carl Zeiss Axioskop 2 microscope (with a 60× plan apo oil immersion objective) and collected with LSM5Pascal 3.2 software (Carl Zeiss Microscopy Ltd, Jena, Germany).

Cell Counting

To determine the degree of eosinophil infiltration in the tissues, 2 of the authors independently manually counted the number of infiltrated cells in 5 random fields using sections stained with hematoxylin and eosin under light microscopy at high magnification (×400) in a blinded manner. The number of mast cells and IgE-positive cells was counted in the same manner using sections that were immunostained for each cell type. This analysis was performed for each sub-region (ie, epithelium, glands, and submucosa).

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, California). The mRNA of the proteases mast cell tryptase and chymase were analyzed using the SYBR Green detection system. The primers and probes for these proteases were designed by SABiosciences (Hilden, Germany). The primers and probes for human β -actin and IL-5 were designed by PE Applied Biosystems, and their mRNA expression was analyzed using the TaqMan detection system. Nuclease-free water was substituted for cDNA in negative controls.

For relative quantification, data were analyzed by the $\Delta\Delta C_t$ method and normalized to the average of housekeeping genes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH)¹⁷ in the SYBR Green detection system and human β -actin in the TaqMan detection system. For each sample using the SYBR Green detection system, the differences in threshold cycles between the proteases and GAPDH or human β -actin genes (ΔC_t sample, ΔC_t control) were determined, and a calibrated ΔC_t value ($\Delta\Delta C_t$, ΔC_t sample minus ΔC_t control) was calculated. Then, the relative quantitation (RQ) values were calculated using the following equation: $RQ = 2^{-\Delta\Delta C_t}$.

Statistical Analyses

Statistical analyses were carried out using SPSS statistical software (SPSS, Inc, Chicago, Illinois). All data are expressed as mean $\pm$ SE in each group. When comparisons were made between groups, the Kruskal-Wallis test was used to assess significant intergroup variability. The significance of the difference between groups was determined using the Mann-Whitney *U* test. Simple linear regression analyses were performed to evaluate the association of each parameter with the Lund-Mackey score based on the assumption that the score reflected the severity of CRSwNP. A difference was considered significant at a *P* value less than .05.

Results

Patient Profiles

Sinonasal and polyp tissues were collected from 17 subjects with ECRS, 17 subjects with non-ECRS, and 7 control subjects. Subject characteristics are presented in Table 1.

Although the review of the database in our hospital identified 12 women with ECRS, all had used topical or oral steroids within 1 month before surgery and therefore were excluded from the present study. Because all patients with aspirin-induced asthma and ECRS in our hospital were using oral steroids before surgery, these patients were excluded from the study. Allergic rhinitis was diagnosed by a radioimmunosorbent assay. Eight of 17 patients

with ECRS and 4 of 17 patients with non-ECRS took the blood radioimmunosorbent assay test and all patients had positive results for allergens.

Real-Time Quantitative PCR Analysis for IL-5, Mast Cell Tryptase and Chymase

Differences in the expression of mast cell tryptase and chymase were assessed at the mRNA level in ECRS polyps, non-ECRS polyps, and non-CRS control specimens (Fig 1). Tryptase expression was significantly higher in ECRS polyps ($P < .05$) and non-ECRS polyps ($P < .01$) than in control specimens but was not significantly different between ECRS polyps and non-ECRS polyps (Fig 1A). In the non-ECRS group, we found a significantly higher mRNA expression of chymase compared with the ECRS group ($P < .05$), but no significant difference from the control group (Fig 1B). In the ECRS group, we found a significantly higher mRNA expression of IL-5 compared with non-CRS controls ($P < .005$) and the non-ECRS group ($P < .005$).

Immunohistochemical Localization of MC-T and MC-TC

To further characterize the mode of mast cell infiltration in CRSwNP, we first performed anti-mast cell tryptase (pan-mast cell marker) immunohistochemistry on ECRS polyps, non-ECRS polyps, and non-CRS control specimens. Consistent with the real-time PCR data, increased numbers of tryptase-positive cells were found in ECRS polyps ($P < .05$) and non-ECRS polyps ($P < .05$; Fig 1D). When examining the regional distribution of mast cells in the mucosa, mast cell infiltration was significantly increased in the epithelium and glands of ECRS polyps compared with control samples (epithelium, $P < .005$; glands, $P < .001$) or non-ECRS polyps (epithelium, $P < .01$; Figs 1E,G and 2). We did not observe a significant difference in the number of mast cells in the submucosa

(excluding those within the glands) between ECRS polyps and controls (Figs 1F and 2).

To assess the distribution of mast cell subtypes in nasal polyps, we used double immunostaining for tryptase and chymase and counted the number of mast cells doubly positive for tryptase and chymase (MC-TC) and singly positive for tryptase (MC-T) in the epithelium, glands, and submucosa of the ECRS and non-ECRS polyps. As presented in Figure 3, of all tryptase-positive mast cells in the epithelium of ECRS polyps, most were MC-T (median 84.9%, interquartile range [IQR] 82.5–97.3; Fig 3A) rather than MC-TC. Also, most tryptase-positive mast cells within the epithelium of non-ECRS polyps were MC-T (median 83.5%, IQR 53.8–90.6; Fig 3D).

As shown in Figure 3, mast cells in the glands of ECRS polyps were mainly MC-T (median 66.8%, IQR 36.6–81.2; Fig 3B). In contrast, most mast cells within the glands of non-ECRS polyps were MC-TC (median 100%, IQR 92.9–100; Fig 3E). Most mast cells within the submucosa of ECRS and non-ECRS polyps also were MC-TC (ECRS, median 93.3%, IQR 89.6–95.4; Fig 3C; non-ECRS, median 97.7%, IQR 95.6–98.2%; Fig 3F).

After comparing the CRS subgroups, the number of MC-T cells was significantly larger in the epithelium, glands, and submucosa of ECRS polyps compared with non-ECRS polyps (Fig 4A). In contrast, the number of MC-TC was significantly larger in the glands and submucosa of non-ECRS polyps compared with ECRS polyps (Fig 4B).

IgE-Positive Cells

The number of cells positive for IgE was counted using immunohistochemical staining in ECRS polyps, non-ECRS polyps, and control specimens. Typical photomicrographs of immunohistochemically stained nasal tissue are shown in Figure 5. The number of IgE-positive cells was significantly larger in the epithelium, glands, and submucosa of ECRS polyps compared with non-ECRS polyps ($P < .001$) and control specimens ($P < .001$; Fig 6A).

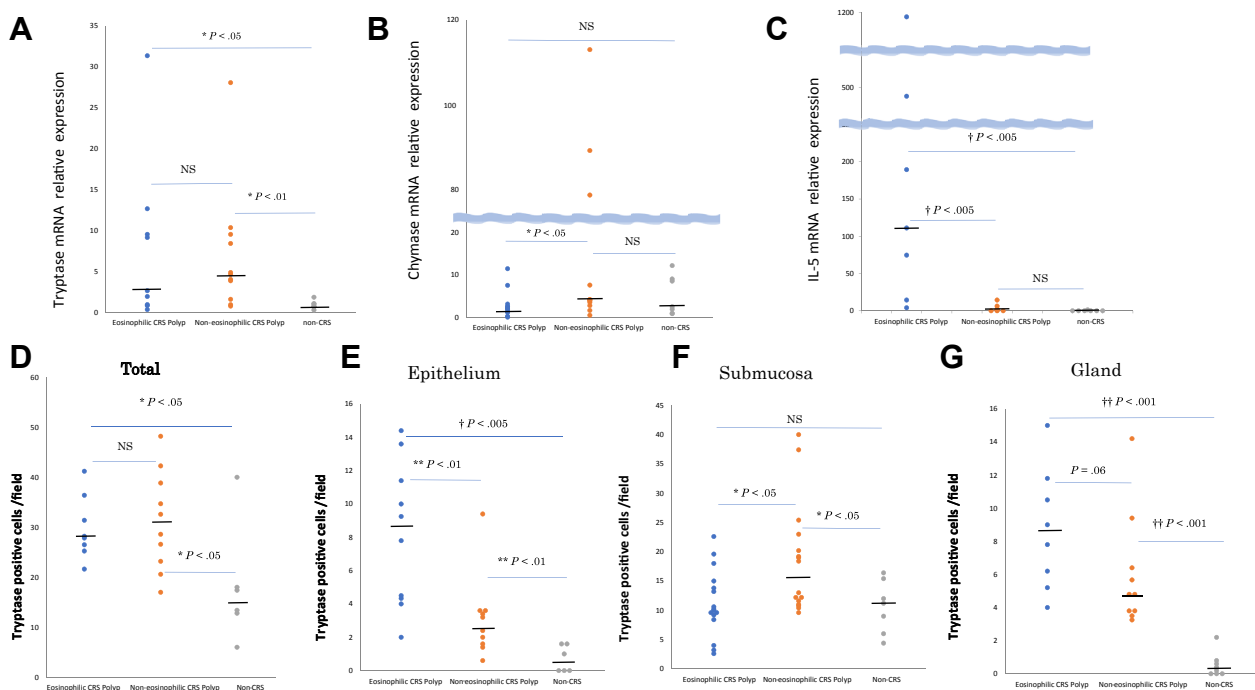


Figure 1. Messenger RNA expression of mast cell tryptase (A), mast cell chymase (B), and interleukin-5 (C) in eosinophilic chronic rhinosinusitis (CRS) polyps, non-eosinophilic CRS polyps, and non-CRS control specimens. Quantitation of mast cell tryptase-positive cells in nasal mucosal tissue from eosinophilic CRS polyps, non-eosinophilic CRS polyps, and non-CRS control specimens and distribution in total mucosa (D), epithelium (E), submucosa (F), and glands (G). Bar, median. * $P < .05$, ** $P < .01$, † $P < .005$, †† $P < .001$. NS, not significant.

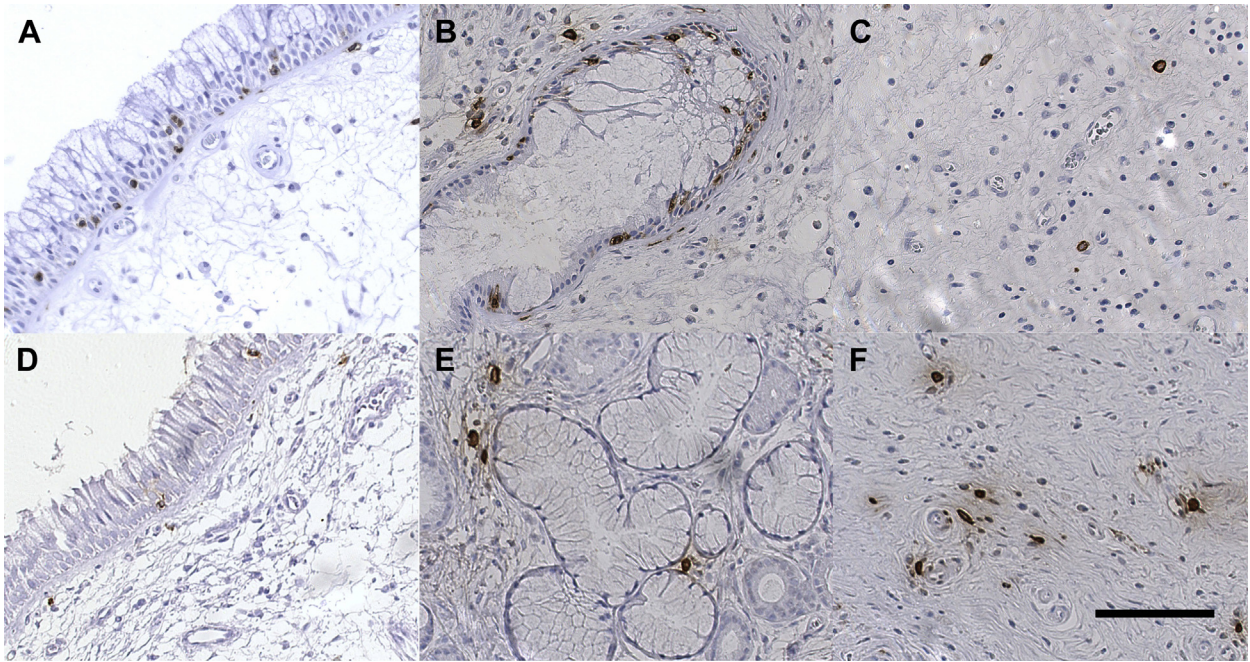


Figure 2. Immunohistochemical detection of tryptase-positive mast cells using anti-human tryptase monoclonal antibody in eosinophilic chronic rhinosinusitis (A–C) and non-eosinophilic chronic rhinosinusitis (D–F) polyps. Immunoreactivity for tryptase is visualized in brown. Representative microscopic views of the epithelium (A, D), glands (B, E), and submucosa (C, F) are shown. Scale bar = 100 μ m.

The identity of IgE-positive cells in the epithelium, glands, and submucosa in nasal polyps was examined using double immunofluorescent staining (Fig 7). IgE in mast cells (mast cell tryptase-positive) was localized to the membranous rim, suggesting IgE receptor expression at the cell surface. The fraction of double-positive cells in mast cell tryptase-positive cells was significantly

larger in the ECRS group in the epithelium, glands, and submucosa compared with the non-ECRS group ($P < .005$; Fig 6B).

Other than the IgE-positive mast cells, we also observed IgE-positive cells whose cell morphology resembled that of plasma cells or eosinophils, as reported in our previous study (data not shown).⁴

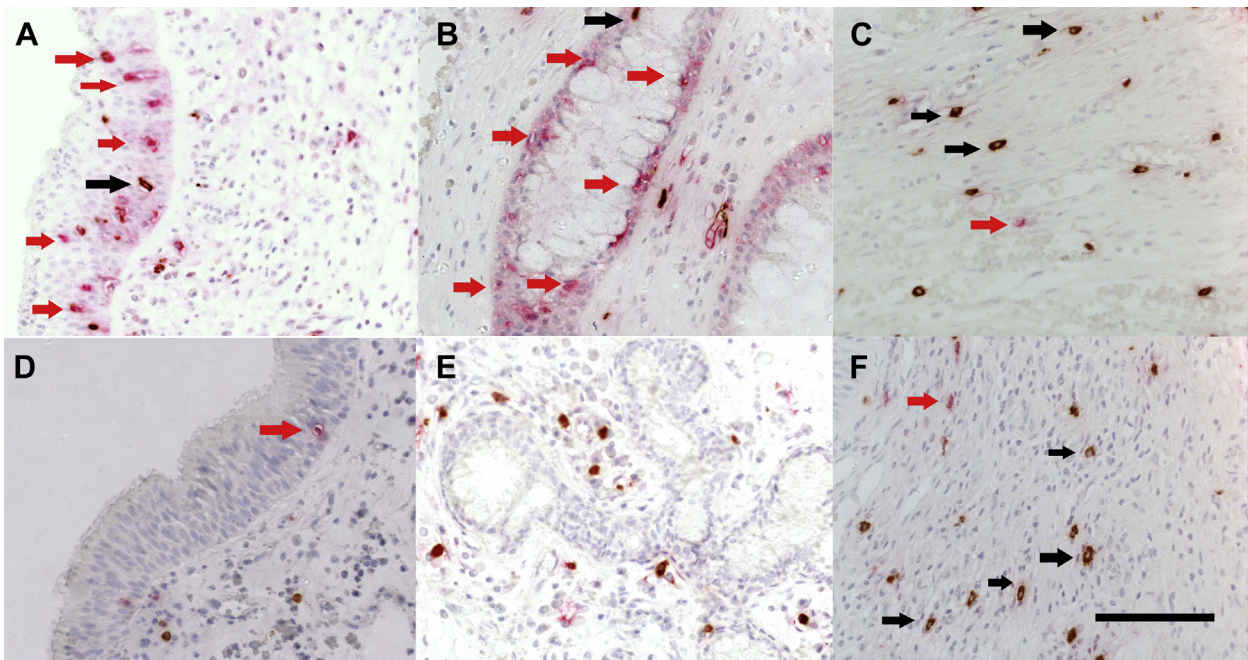


Figure 3. Double-immunohistochemical staining for tryptase and chymase in eosinophilic chronic rhinosinusitis (A–C) and non-eosinophilic chronic rhinosinusitis (D–F) polyps. Images show representative microscopic views of the epithelium (A, D), glands (B, E), and submucosa (C, F). Immunoreactivity for tryptase is visualized in red and immunoreactivity for chymase is visualized in brown. Some examples of single-positive tryptase cells (red arrows) and double-positive cells (black arrows) are marked. Scale bar = 100 μ m.

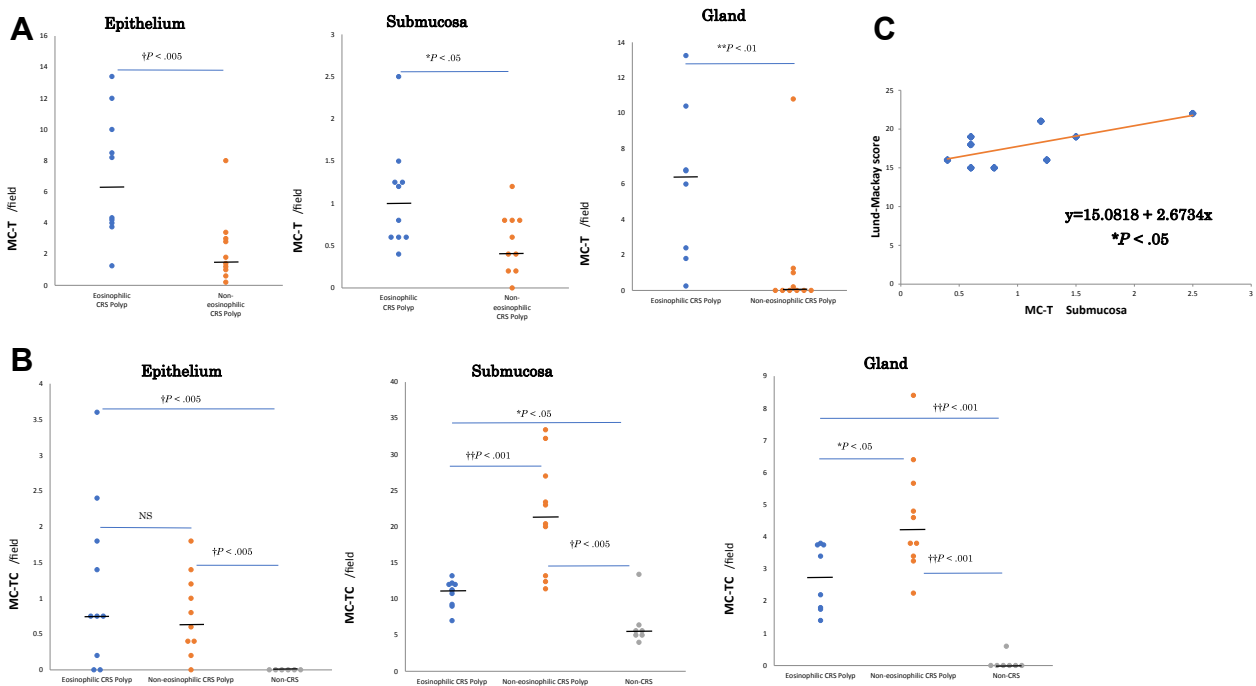


Figure 4. Quantitation of mast cell tryptase-positive (MC-T) (A) and mast cell tryptase- and chymase-positive (MC-TC) (B) cells in nasal mucosal tissue from eosinophilic chronic rhinosinusitis (CRS) polyps, non-eosinophilic CRS polyps, and non-CRS control specimens and distribution in epithelium, submucosa, and glands. We could not perform double immunostaining for mast cell tryptase and chymase in non-CRS specimens because of a lack of samples. (C) Results of simple linear regression analysis between the submucosal MC-T count in eosinophilic CRS nasal polyps and the Lund-Mackay score are shown. Bar, median. $* P < .05$, $** P < .01$, $\dagger P < .005$, $\dagger\dagger P < .001$. NS, not significant.

Association Between Immunohistochemical Analysis and Clinical Severity

In simple linear regression analyses, only the submucosal MC-T count in ECRS nasal polyps correlated with the Lund-Mackay score. IL-5 mRNA, eosinophil count in nasal tissue, and

immunohistochemical counts (IgE, MC-TC, epithelial and gland MC-T) did not correlate with the Lund-Mackay score. Also, IgE-positive cell, MC-T, and MC-TC counts did not correlate with the IL-5 mRNA or eosinophil count of nasal tissue in patients with ECRS. In non-ECRS nasal polyps, IgE-positive cell, MC-T, and MC-TC counts

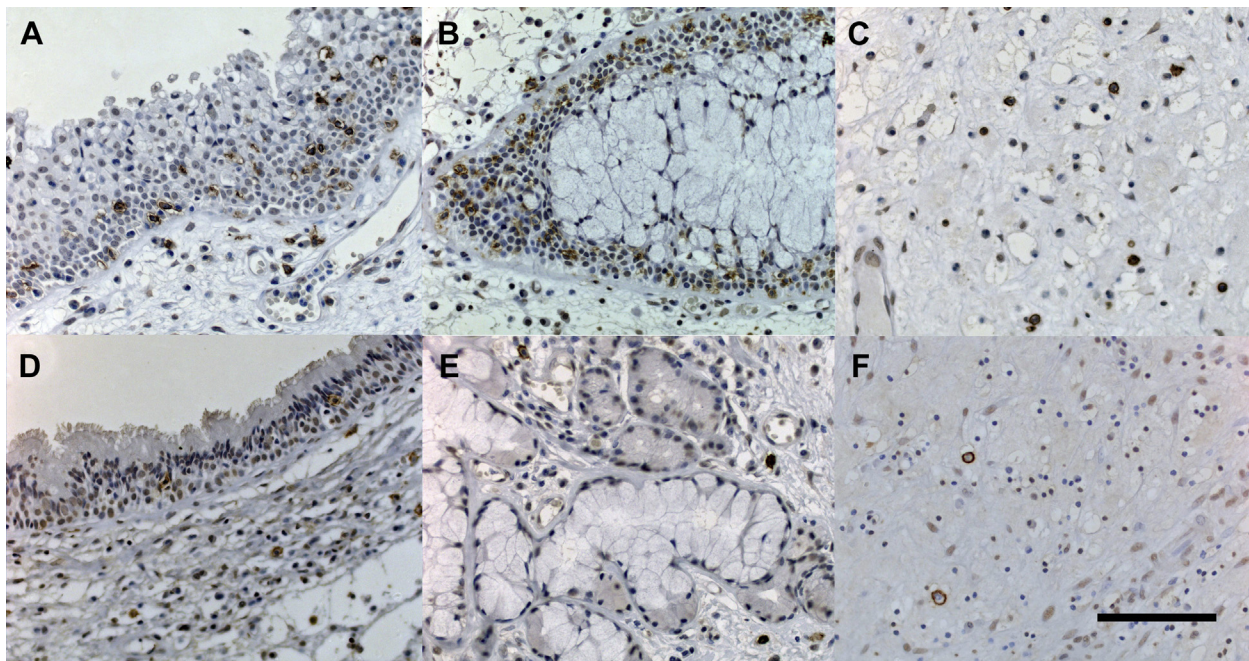


Figure 5. Immunohistochemical staining for immunoglobulin E using anti-immunoglobulin E antibody in eosinophilic chronic rhinosinusitis (A–C) and non-eosinophilic chronic rhinosinusitis (D–F) polyps. Immunoreactivity for immunoglobulin E is visualized in brown. Representative microscopic views of the epithelium (A, D), glands (B, E), and submucosa (C, F) are shown. Scale bar = 100 μ m.

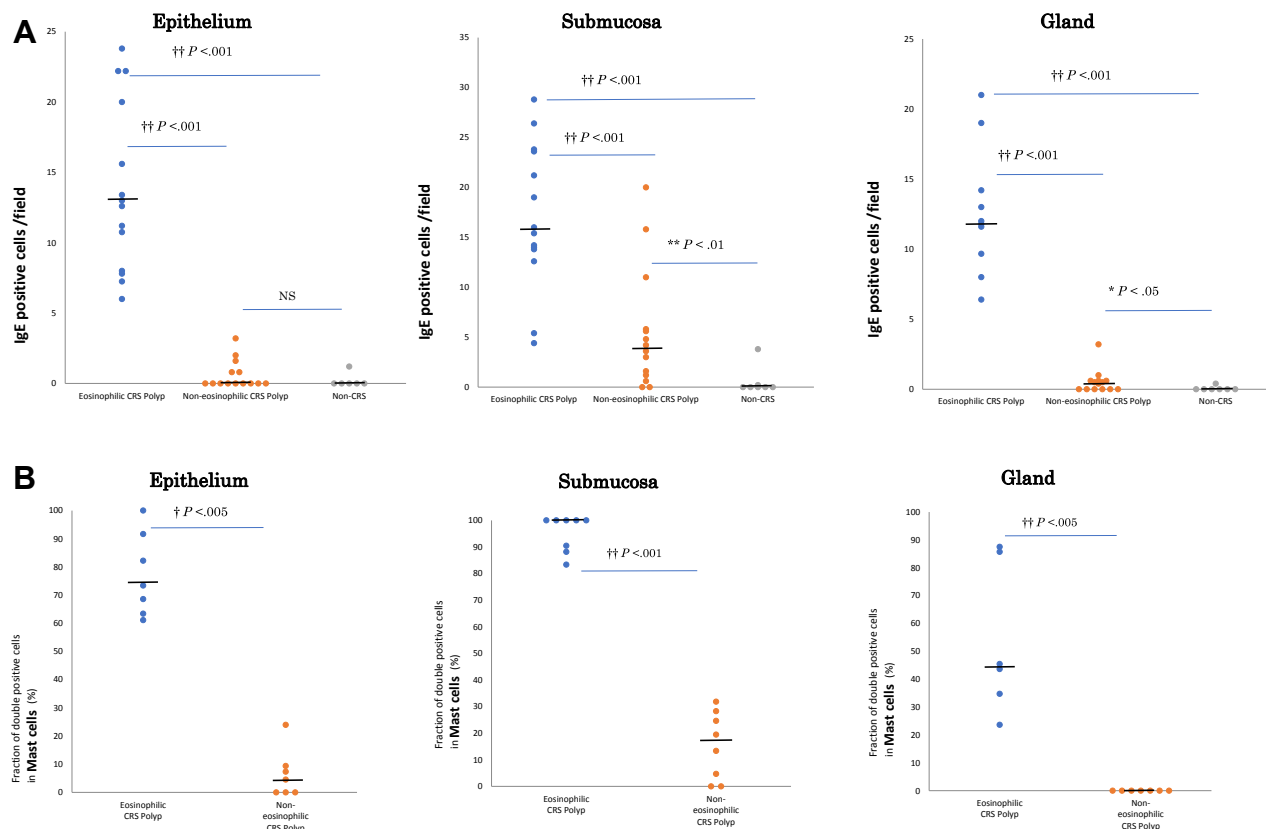


Figure 6. (A) Quantitation of immunoglobulin E (IgE)-positive cells in nasal mucosal tissue from eosinophilic chronic rhinosinusitis (CRS) polyps, non-eosinophilic CRS polyps, and non-CRS control specimens and distribution in the epithelium, submucosa, and glands. (B) The fraction of double-positive cells in IgE and mast cell tryptase-positive cells in nasal mucosal tissue from eosinophilic CRS polyps, non-eosinophilic CRS polyps, and non-CRS control specimens and distribution in the epithelium, submucosa, and glands. Bar, median. * $P < .05$, ** $P < .01$, † $P < .005$, †† $P < .001$.

did not correlate with the Lund-Mackay score, IL-5 mRNA, or eosinophil count in nasal tissue. The results of the simple linear regression analysis between the submucosal MC-T count and the Lund-Mackay score in ECRS nasal polyps are shown in Figure 4C.

Discussion

In this study, we have shown that the number of mast cells and the expression of MC-T mRNA were increased in ECRS and non-ECRS polyps in Japanese patients with CRSwNP. This finding is in line with previous analyses showing that the amount of mast cell infiltration is increased in nasal polyps of patients with CRSwNP in various ethnic groups.^{9,12,13}

Our analysis further demonstrated that, although the number of mast cells was increased in nasal polyps, the distribution and subtype population of these mast cells were different between ECRS and non-ECRS polyps. The increased infiltration of mast cells was localized to the epithelium and glands in ECRS polyps, whereas mast cell infiltration was mainly confined to the submucosa in non-ECRS polyps. The mast cells found in the epithelium and glands of ECRS polyps were predominantly of the MC-T subtype. In contrast, mast cells in the glands and submucosa of non-ECRS polyps were mainly of the MC-TC subtype. These observations suggest that mast cell infiltration might be involved in the pathogenesis of CRSwNP in a different way between the ECRS and non-ECRS types.

In contrast to our result, Takabayashi et al¹² reported that glandular mast cells showed MC-TC phenotypes in American patients with CRSwNP who are considered to be of the ECRS

phenotype. The discrepancy regarding the phenotype of glandular mast cells between the studies could be associated with the ethnic background of the patient population. Alternatively, it could be related to the clinical severity of the CRS. Balzar et al¹⁸ reported that in patients with asthma, the phenotype distribution of MC-T and MC-TC in the bronchial mucosa alters depending on the severity of asthma. Similar differences in the phenotype distribution of mast cells also might occur in cases of ECRS.

Recent studies have demonstrated the biological difference between MC-T and MC-TC. For example, MC-TC cells express CD88, the receptor for C5a, and therefore degranulate in response to C5a, whereas MC-T cells do not express CD88 or respond to C5a.¹⁹ Also, MC-TC cells are more resistant to corticosteroid therapy than MC-T cells.^{18,20,21} How these biological differences and the differential distribution of MC-T and MC-TC in ECRS and non-ECRS nasal polyps are involved in the pathogenesis of each type of CRSwNP remains unclear and needs further investigation. The predominance of MC-TC in non-ECRS polyps could be associated with the steroid-resistant characteristics of non-ECRS polyps. We also found that the submucosal MC-T count in nasal polyps correlated with the Lund-Mackay score in patients with ECRS. Peric et al²² indicated a correlation between the Lund-Mackay score and the level of cytokines, such as IL-4, in the nasal secretions of patients with CRSwNP. Furthermore, Kagoya et al²³ reported that the basophil count in nasal polyps correlated with the Lund-Mackay score in patients with ECRS. Our results agree with their results in showing that biomarkers of T-helper cell type 2 inflammation were associated with the severity of the Lund-Mackay score.

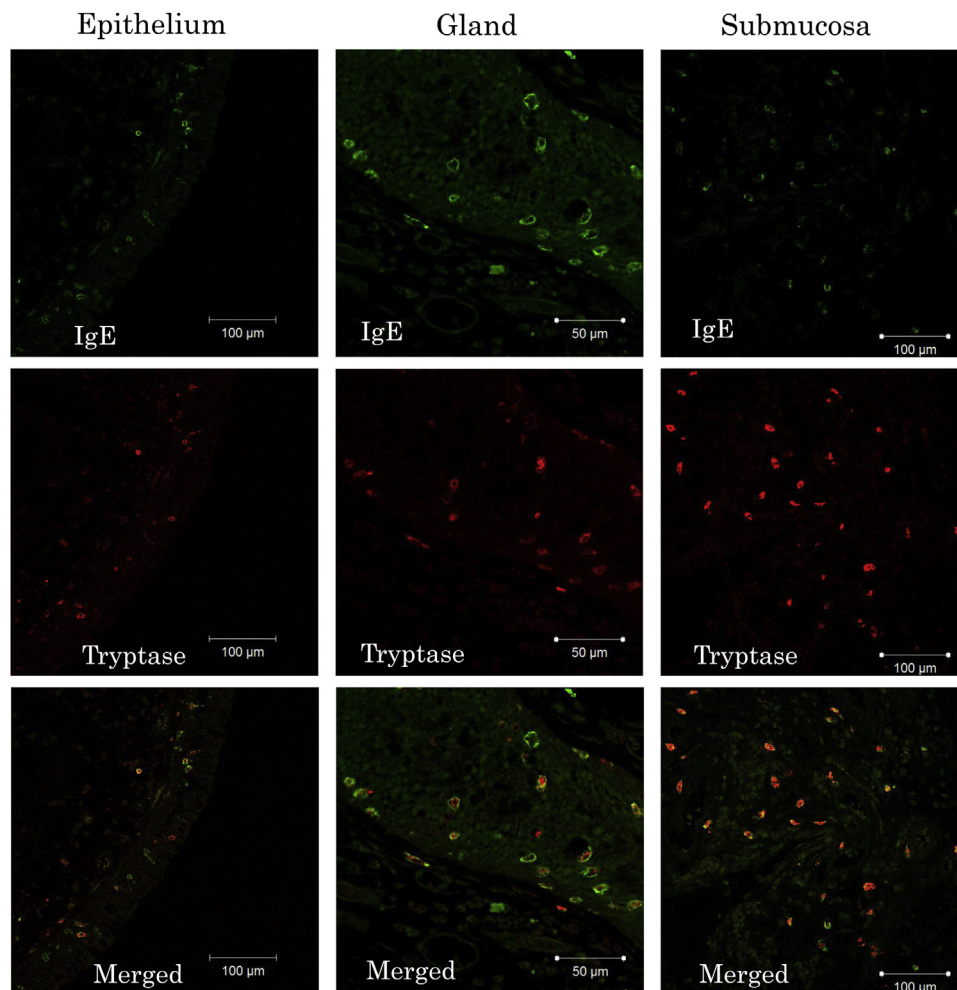


Figure 7. Double-immunohistochemical staining for immunoglobulin E (IgE) mast cell tryptase in eosinophilic chronic rhinosinusitis polyps. Immunoreactivities for IgE and mast cell tryptase are visualized in green and red, respectively. Representative microscopic views of the epithelium (left), glands (center), and submucosa (right) are presented.

In this study, we could not examine allergy test results for all participants. Therefore, it remains unclear whether comorbid type I allergy correlates with the distribution, subtype population, and IgE positivity of mast cells in CRS polyps. Further study is necessary to address this issue.

Our study also demonstrated that IgE-positive mast cells were significantly increased in the epithelium, glands, and submucosa of ECRS polyps compared with non-ECRS polyps. The proportion of IgE-tryptase double positivity in total tryptase-positive cells was 73.4% in the epithelium and nearly 100% in the submucosa. These data indicate that MC-T (the dominant phenotype in the epithelium) and MC-TC (the dominant phenotype in the submucosa) bind IgE in ECRS polyps. It has been shown that MC-T and MC-TC respond when stimulated with the high-affinity IgE receptor FcεRI.¹⁹ Thus, MC-T and MC-TC could be involved in IgE-mediated mast cell activation in the pathogenesis of ECRS. Further investigation on the mode of chemical mediator release in MC-T and MC-TC is necessary to understand the role of these mast cell subtypes in the pathogenesis of ECRS.

Acknowledgments

We thank Sayaka Igarashi, Yukari Kurasawa, Atsuko Tsuyuzaki, and Kimiko Miwa for technical assistance.

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