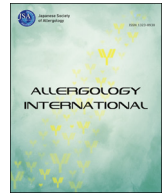




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Invited Review Article

Eosinophilic chronic rhinosinusitis

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ABSTRACT

Eosinophilic chronic rhinosinusitis (ECRS) is a subgroup of chronic rhinosinusitis with nasal polyps (CRSwNP), which is associated with severe eosinophilic infiltration and intractable. Its symptoms include dysosmia, nasal obstruction, and visous nasal discharge. The cause of ECRS is not clear, although it is thought that *Staphylococcus aureus* and its enterotoxins are involved in stimulating the Th2 system to promote IgE production and eosinophil infiltration through various pathways. While, the coagulation system is activated and the fibrinolytic system is suppressed, leading to deposition of fibrinous networks in nasal polyps. Therefore, a fibrin-degrading agent could be a new treatment for ECRS.

Genetic analysis of nasal polyp cells using next-generation sequencing has identified some of the factors involved in ECRS, including periostin, which can be used as a biomarker of this condition. A protease inhibitor could be a therapeutic agent for ECRS. Regarding the role of eosinophils, many researchers have been interested in the mechanism of ETosis. However, the mechanism leading to development of nasal polyps is unknown.

In Japan (as well as in East Asia), the incidence of non-ECRS is decreasing and that of ECRS is increasing, but the reason is also unknown. Thanks to the development of biologics therapy, it is thought that there will be a shift to precision medicine in the future.

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History of chronic rhinosinusitis in Japan

It was thought that the incidence of rhinosinusitis was higher in Japan than in Europe or the United States because Japanese people have flatter faces than Europeans and Americans. The pathogenesis of chronic rhinosinusitis (CRS) involves production of secretions in the paranasal sinuses in response to viral infection and secondary bacterial infection, followed by accumulation of these secretions in the sinuses due to impaired drainage and development of persistent inflammation. Patients with CRS complain of symptoms such as purulent nasal discharge, nasal obstruction, headache, cheek pain, and toothache. In Japan, this condition was often known as empyema, rather than CRS. In some patients with CRS, polyps develop in the nasal cavity, while other patients do not have any polyps. CRS associated with nasal polyps is called CRS with nasal

polyps (CRSwNP), while the other type is called CRS without nasal polyps (CRSSNP).

After World War II in Japan, use of the Caldwell-Luc operation under local anesthesia to treat CRS was popularized by otorhinolaryngologists, including those with their own clinics. In this operation, purulent fluid is aspirated from the maxillary sinus, the affected mucous membrane is removed, and a nasoantral window is made for drainage. However, the Caldwell-Luc operation only achieved a cure rate of 60%, so further improvement was required.¹ In 1985, Kennedy² and Stammberger³ reported endoscopic sinus surgery (ESS) for CRS, and it was soon performed widely in Japan. After the efficacy of long-term low-dose macrolide therapy for CRS was reported, treatment of CRS in Japan changed dramatically.^{4,5} In particular, the combination of ESS and postoperative macrolide therapy considerably improved the outcome.

However, CRS in Japan seemed to differ from that in Europe and the United States. In 1962, Okuda reported that CRSwNP in Europe and the United States showed eosinophil-dominant inflammatory cell infiltration, while neutrophil-dominant infiltration was observed in Japan. Okuda obtained these findings by comparing nasal polyps between European patients and Japanese patients while studying in Austria.⁶ Subsequently, many reports were

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published that confirmed the existence of neutrophil-dominant inflammatory cell infiltration in Japanese CRS. As mentioned above, long-term low-dose macrolide therapy was the first treatment for CRS to demonstrate efficacy in Japan. This treatment method was originally approved for diffuse pulmonary bronchiolitis with neutrophil-dominant inflammatory cell infiltration.⁷ Diffuse pulmonary bronchiolitis is often associated with CRS, and this combination is known as the sinobronchial syndrome. When CRS with neutrophil-dominant inflammatory cell infiltration was treated by macrolide therapy for diffuse pulmonary bronchiolitis with neutrophil-dominant infiltration, excellent results were achieved.⁴ Now, macrolide therapy is also widely used in Europe and the United States, and is becoming first-line treatment for CRSsNP.⁸

Appearance of eosinophilic CRS (ECRS) and establishment of diagnostic criteria

Since the late 1990s, there has been an increase of patients in whom nasal polyps recur soon after removal by ESS combined with postoperative macrolide therapy. A survey of such patients showed that dysosmia was their chief complaint and they also had viscous nasal discharge and nasal obstruction. Eosinophil-dominant inflammatory cell infiltration was observed in nasal polyp tissues obtained from these patients, instead of the previously common pattern of neutrophil-dominant infiltration. Other differences between these patients and ordinary CRS patients were also noted, e.g., bronchial asthma was a frequent complication in the patients with eosinophil-dominant inflammatory cell infiltration (Table 1). Therefore, intractable CRSwNP was named eosinophilic CRS (ECRS).⁹ However, the diagnostic criteria for ECRS were initially vague, and it was diagnosed if a patient had symptoms and histopathological examination showed eosinophil-dominant inflammatory cell infiltration. In order to establish guidelines for the diagnosis of ECRS, a multicenter large-scale epidemiological study “Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis Study” (JESREC Study) was initiated in 2010 as a part of the Research on Measures for Intractable Diseases by the Japanese Ministry of Health, Labour and Welfare. Eventually, diagnostic criteria for ECRS were established, as shown in Table 2. Using these diagnostic criteria, the JESREC score is determined as the sum of the scores for four items and a diagnosis of ECRS is made if the JESREC score is 11 points or higher.¹⁰ A final diagnosis of ECRS is made by examination of a biopsy specimen or resected tissue under a microscope at 400× (ocular lens, field number 22). When the mean number of eosinophils in three fields is 70 or more, a definite diagnosis of ECRS can be made.

Table 1
Difference between ECRS and CRSwNP neutrophil type.¹⁰

	ECRS	CRSwNP neutrophil type
Age	20 years or older	All ages
Main symptoms	Dysosmia	Nasal obstruction Nasal discharge Headache
Properties of nasal discharge	Viscous, colloidal	Purulent, mucous
Features of nasal polyps	Bilateral & multiple	Unilateral or bilateral & solitary or multiple
Site of nasal polyps	Middle meatus & Olfactory cleft	Middle meatus
Main site involved	Ethmoid sinus	Maxillary sinus
Predominant infiltrating cell	Eosinophil	Neutrophil
Complications	Asthma Aspirin intolerance Drug allergy	Diffuse bronchiolitis Bronchiectasis
Oral steroid treatment	Effective	Resistance
Macrolide therapy	Resistance	Effective

Table 2
Criteria for diagnosis of eosinophilic chronic rhinosinusitis (JESREC score).¹⁰

Factor	Score
Side affected: both sides	3 points
With nasal polyps	2 points
CT changes: ethmoid/maxillary ≥ 1	2 points
Peripheral blood eosinophil count (%)	
2 < and $\leq 5\%$	4 points
5 < and $\leq 10\%$	8 points
10% <	10 points

When the total score is 11 points or higher, the possibility of eosinophilic chronic rhinosinusitis is high. A definite diagnosis of eosinophilic chronic rhinosinusitis should be made by microscopic examination of nasal polyp/paranasal sinus tissues, if three fields inspected under a microscope at a total magnification of 400× (ocular lens, field number 22) show 70 or more eosinophils per field.

Next, criteria for determining the severity of ECRS were defined, with two factors being assessed to determine the severity of ECRS. The first factors are a peripheral blood eosinophil percentage of more than 5% and ethmoid sinus-predominant involvement on CT scans. The second factor is the presence of complications (current asthma or a history of asthma, aspirin intolerance, and/or NSAID allergy). The algorithm is displayed in Figure 1. Eventually, CRS was classified into the following four categories: non-ECRS, mild ECRS, moderate ECRS, and severe ECRS. After treatment of ECRS for six years, Kaplan–Meier analysis showed that the frequency of recurrent nasal polyps was 13% in patients with non-ECRS, 23% in those with mild ECRS, 31% in those with moderate ECRS, and 52% in those with severe ECRS, with significant differences among these four groups.¹⁰

Chronic rhinosinusitis in Europe and the United States

In Europe and the United States, chronic rhinosinusitis is only classified into two categories, which are CRSsNP and CRSwNP. About 20% of patients with CRS are diagnosed as having CRSwNP and require ESS.¹¹ In European and American patients with CRSwNP, eosinophil-dominant inflammatory cell infiltration is generally observed and CRSwNP is often complicated by asthma. Accordingly, it is thought that CRSwNP is caused by a type 2 immune response. In contrast, CRSsNP is thought to be caused by a type 1 immune response to infection, since neutrophil-dominant inflammatory cell infiltration is generally observed.¹²

It has been reported that eosinophil-dominant inflammatory cell infiltration is found in 60–90% of CRSwNP patients from Europe and the United States, although the definition of eosinophil-dominant infiltration used in that study was different from the Japanese definition and only required the presence of 5–10 or more eosinophils per field (total magnification of 400×) for classification as eosinophil-dominant.¹¹ On the other hand, the threshold is much higher in Japan and the number of eosinophils per field (total magnification of 400×) needs to be 70 or more or 200 or more to be considered eosinophil-dominant.^{13–15} In American patients with CRSwNP undergoing ESS, recurrence was observed at 18 months postoperatively in 40% of patients.¹⁶ In that study, 56% of the patients also had asthma as a complication, which was a much higher frequency than the asthma rate of 27% for the ECRS group in the JESREC study.

Bachert's group from Ghent University (Belgium) defined three phenotypes of CRS (Fig. 2).¹⁷ Non-type 2 group had CRSsNP with little asthma comorbidity. The moderate type 2 group indicated CRSwNP with increase asthma prevalence. Severe type 2 group was associated with CRSwNP in nearly all cases and asthma comorbidity in up to 70%. These findings suggest that mild ECRS and moderate

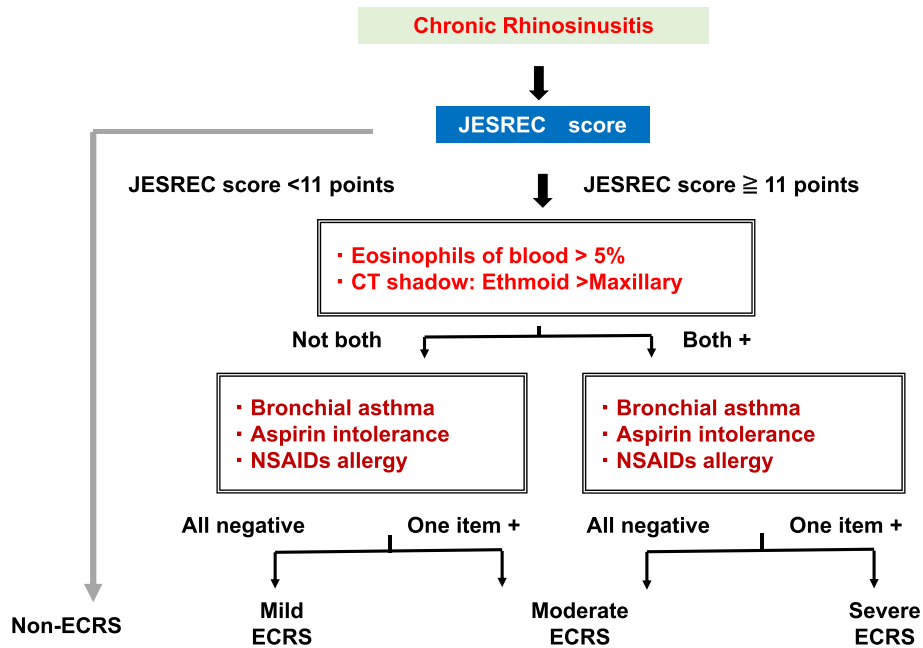


Fig. 1. Diagnostic algorithm for refractory ECRS.¹⁰ Patients with CRS would be classified into four categories by JESREC score, Factors A and B. Factor A is a peripheral blood eosinophil percentage >5% and ethmoid-dominant changes on CT. Factor B is comorbidities (asthma, aspirin intolerance, and NSAID intolerance). Factor A (+): both of two factors fulfilled, (-): at least one factor not fulfilled. Factor B (+): at least one factor fulfilled, (-): all three factors not fulfilled.

ECRS in Japan are similar to moderate type 2 group in Europe. Severe ECRS in Japan is also similar to severe type 2 group in Europe (Fig. 2).

Is ECRS hereditary?

There have not been any reports that ECRS has an obvious familial tendency. Recently, Kristjansson *et al.* published an interesting genome-wide association study of patients with nasal polyps, patients with CRS, and controls, which showed that CRSwNP were associated with mutations of ALOX15, HLA, IL-18R1-IL1RL1, IL-33, FOXP1, CYP2S1, SLC22A4, MYRF, TSLP, and 10p14.¹⁸ They found that nasal polyps were not likely to arise when there was no 15-LO enzyme activity due to a missense variant of ALOX15. Our SNIP analysis showed that ECRS may be associated with the rs1837253 genotype in TSLP, and some of our findings were consistent with the data obtained by Kristjansson and colleagues.¹⁹ Miyata *et al.* reported that metabolic products downstream of ALOX15 were decreased due to abnormal ALOX15 enzyme activity

in eosinophils from nasal polyps, which were inconsistent with data reported by Kristjansson *et al.*²⁰ We compared RNA expression between nasal polyps and inferior turbinate mucosa by RNAseq analysis (data shown below), and showed that ALOX15 expression was significantly increased in nasal polyps.²¹ Accordingly, we have no doubt that ALOX15 is involved in ECRS to some extent.

Does *Staphylococcus aureus* cause ECRS?

As is the case with allergic rhinitis (AR), it is thought that ECRS and CRSwNP develop in a Th2-dominant environment.²² In patients with AR, allergens including pollens and mites enter the nasal mucosa, where dendritic cells capture the allergens and transmit information to naive CD4-positive T cells, which differentiate into Th2 cells under stimulation by IL-4 to produce IL-4, IL-5, and IL-13. It has been reported that IL-4 stimulates differentiation of B cells into allergen-specific IgE-producing plasma cells, which then produce IgE. The allergens associated with ECRS have not been clarified (Fig. 3). Bacteria are potential candidate allergens. When

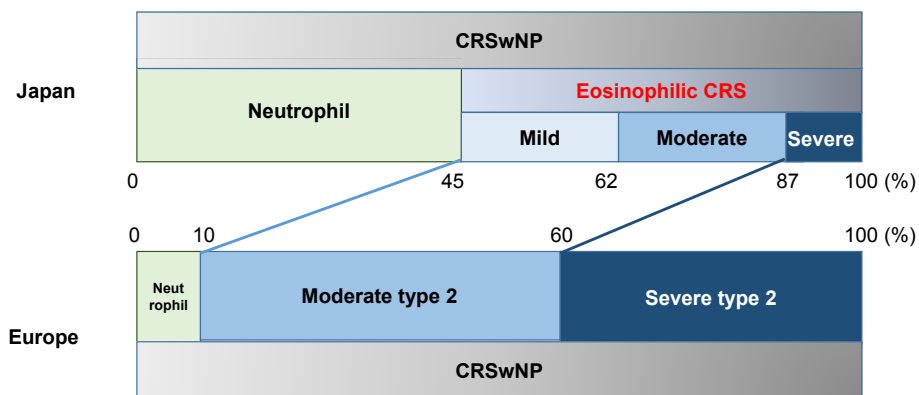


Fig. 2. Phenotype of CRSwNP: Comparison between Japan and Europe. Mild and moderate ECRS in Japan are similar to moderate Type 2 CRSwNP in Europe. Severe ECRS in Japan is also similar to severe type 2 CRSwNP in Europe.

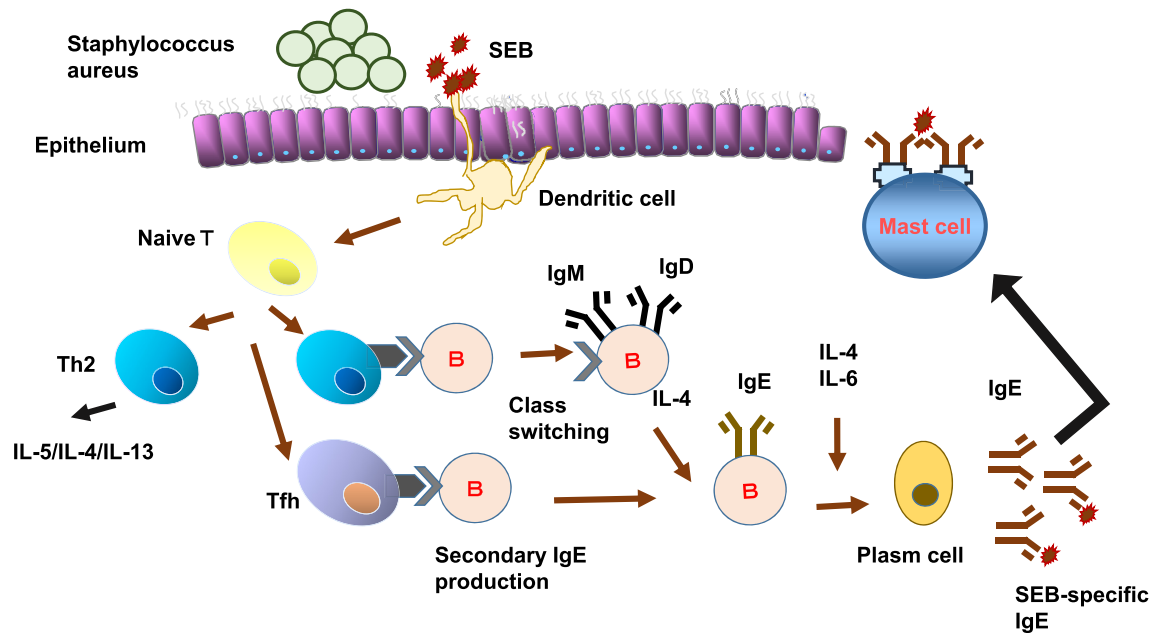


Fig. 3. Mechanism of Staphylococcal enterotoxin-specific IgE production.^{23–25} SEB induces SEB-specific IgE by Th2 cytokine. SEB, Staphylococcal enterotoxin B.

Staphylococcus colony formation in the nasal cavity was examined, it was found that the colony count was higher in patients with CRSwNP than in healthy subjects or patients with CRSsNP. Bachert's group demonstrated that nasal polyps contained Staphylococcal enterotoxin (SE)-specific IgE, which was directed against SEs produced by *S. aureus*, and they suggested that SE was a causative allergen of CRSwNP.²³ SE also plays a role as a superantigen that nonspecifically activates T cells, after which the activated T cells release Th2 cytokines such as IL-4, IL-5, and IL-13. These cytokines activate various types of antigen-specific B cells, which produce polyclonal IgE (Fig. 3). Binding of IgE to mast cells causes the release of chemical mediators (especially eosinophil chemotactic factors) in response to various antigens, resulting in eosinophilic inflammation.

Among the SEs, staphylococcal enterotoxin B (SEB) stimulates the production of IL-5, IL-13, and RANTES by nasal polyp cells, while production of these cytokines in response to SEB is inhibited by prostaglandin E₂ (PGE₂).²⁴ Lipopolysaccharide (LPS) is a cell wall component of gram-negative bacteria that promotes PGE₂ production, thus inhibiting production of IL-5 and IL-13 by nasal polyp cells stimulated with SEB²⁵ (Fig. 4). On the other hand, SEB inhibits the production of PGE₂ and cyclooxygenase 2 (COX-2) by nasal epithelial cells, which means that SEB can prevent suppression of eosinophilic inflammation and promote the persistence of an inflammatory response (Fig. 4). In mice, it was shown that infection with *S. aureus* is prevented by IFN- λ 1.²⁶ When cultured nasal mucosa cells from healthy individuals were infected with *S. aureus*, the number of the infected cells was significantly decreased by addition of IFN- λ 1 to the culture. The IFN- λ 1/IL-28/reactive oxygen species (ROS)/Janus kinase/STAT signaling pathway was involved in prevention of *S. aureus* infection. However, a similar experiment showed that IFN- λ 1 did not decrease the number of nasal polyp cells with *S. aureus* infections, suggesting that there were differences between nasal mucosa cells and nasal polyp cells.²⁷

A recent analysis of extracellular *S. aureus* proteins to which human IgG4 binds identified six Staphylococcal serine protease-like proteins (SplA–F). T cell specific for each Spl showed significant production of IL-4, IL-5, and IL-13. However, the concentrations of Spls required to stimulate production were substantially higher than that of SEB. On the other hand, levels of SplA, SplB,

SplD, and SplE specific IgE were significantly higher in patients with asthma than in healthy individuals. When mice received repeated intratracheal administration of SplD, eosinophilic infiltration was observed in the lungs and SplD-specific IgE was detected in the serum.²⁸ In addition, eosinophilic inflammation caused by SplD was inhibited by blocking IL-33 activity with soluble ST2 receptors, indicating that such inflammation is mediated by the IL-33/ST-2 axis.²⁹

Is *S. aureus* the only causative microorganism?

Infection of nasal polyp tissues with non-enterotoxigenic *S. aureus* increased the production of IL-33, TSLP, IL-5, and IL-13. When inferior turbinate tissues from healthy individuals were infected with non-enterotoxigenic *S. aureus*, only TSLP production was increased. On the other hand, when nasal polyp tissues and inferior turbinate tissues from healthy individuals were infected with *Staphylococcus epidermidis*, there was no increase in the production of any cytokine or factor. These findings clearly showed that *S. aureus* is involved in promoting an increase of cytokine production in epithelial tissue.³⁰

It was thought that certain types of *S. aureus* could disrupt intercellular adhesion factors and damage the epithelial ciliary transport system and barrier function. Interestingly, a recent study showed that expression of intercellular adhesion factors was increased and barrier function was improved by the presence of *S. aureus* in nasal mucosa tissues from healthy subjects, while this effect was not observed and expression of adhesion factors was suppressed in nasal mucosa tissues from patients with CRSwNP.³¹ When primary cultured epithelial cell lines derived from nasal polyps of patients with CRSwNP were exposed to components of *S. aureus*, wound healing was delayed due to delayed repair, reduction of lamellipodial protrusion, and reduction of migratory activity compared with control primary cultured epithelial cell lines.³² In addition, substances in *S. aureus* culture supernatant delayed wound healing by primary cultured epithelial cell lines derived from nasal polyps of CRSwNP patients. On the other hand, treatment of cells with a Rho-associated coiled-coil kinase (ROCK) inhibitor normalized wound healing. These findings suggested that *S.*

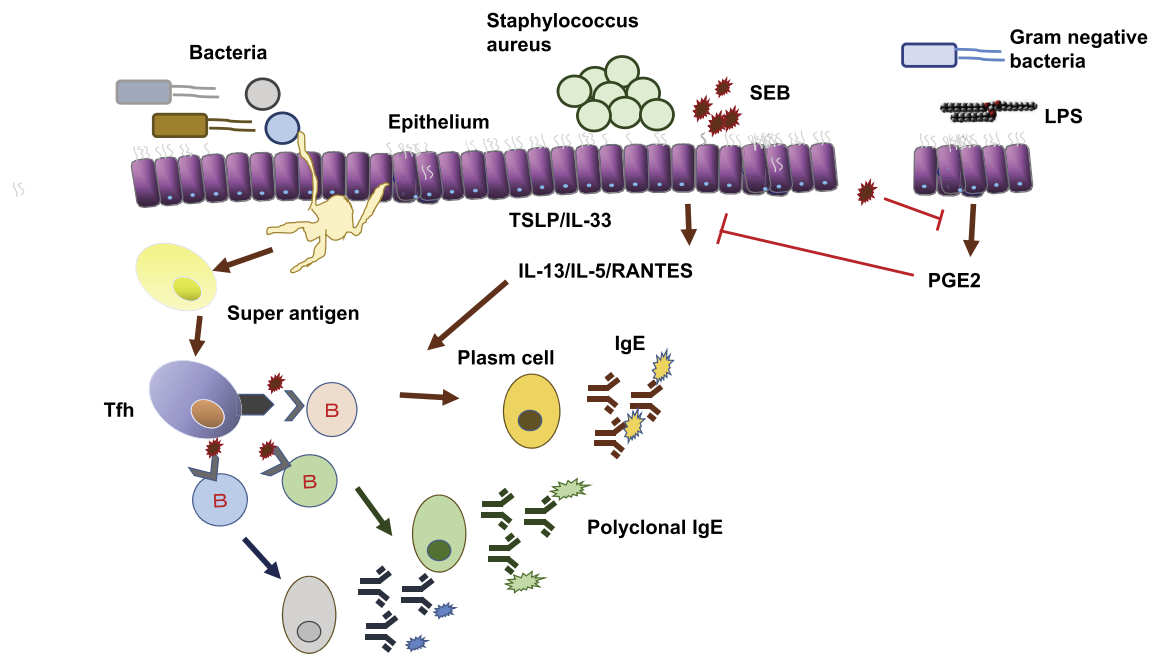


Fig. 4. Mechanism of nonspecific IgE production.^{23,35} SEB and some bacteria induce epithelial and type 2 cytokines. These cytokines induce polyclonal IgE.

aureus and substances in its culture supernatant can delay wound healing in the nasal mucosa by activation of ROCK.

Production of IL-5 was observed in nasal polyp tissues in which *S. aureus* was detected by high performance mass spectrometry, while the IL-5 level decreased after *S. aureus* was killed by an antibacterial agent.³³

IgE class switching can occur in the nasal mucosa.³⁴ Based on the detection of SE-specific IgE in nasal polyps, *S. aureus* is thought to be the causative organism of ECRS. However, it has been shown that IgG, IgA, and IgE directed against *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* are also found in nasal polyp tissues and that Tfh cells play an important role in immunoglobulin class switching (Fig. 4). In addition, it has been reported that existence of bacteria-specific IgE in the nasal cavity can be explained by sequential class switching from bacteria-specific IgG or IgA1.³⁵ Given these findings, it seems probable that several species of bacteria can promote the formation of nasal polyps under certain conditions.

Role of fibrin in eosinophilic chronic rhinosinusitis

We previously found excessive formation of fibrinous deposition in nasal polyps from patients with ECRS.³⁶ Fibrinous networks are formed during the process of wound healing and then are infiltrated by inflammatory cells. Fibrinous networks usually dissolve within a few days and edema improves. However, the coagulation system and/or fibrinolytic system regulate the formation/dissolution of these fibrinous networks, and these systems may be abnormal in the nasal polyps of ECRS patients, with such abnormalities being involved in the development of this disease (Fig. 5). *S. aureus* produces coagulase, which coagulates plasma, and this could also influence the onset of ECRS.

Therefore, we performed screening for expression of all coagulation factor genes and found a marked increase in the expression of coagulation factor XIII-A (FXIII-A) in nasal polyps from ECRS patients.³⁷ FXIII is a transglutaminase that cross-links proteins, and it promotes the formation of hard fibrin clots from fibrinous networks in the final stage of coagulation. We performed

immunohistochemical analysis to determine the localization of FXIII-A in nasal polyps from patients with ECRS, and we found that it was expressed by M2 macrophages infiltrating the submucosal tissues. It was reported that CCL18-expressing M2 macrophages are increased in nasal polyps of patients with ECRS.³⁸ CCL-18 is involved in fibrosis and in the formation of fibrinous networks (data shown below). Additionally, mast cells release eosinophil chemotactic factors and can stimulate hyperfunction of coagulation system. Three types of mast cells have been identified in ECRS (tryptase, chymase, carboxypeptidase A3), which are different from those seen in healthy persons, but the role of these mast cells in ECRS is unknown.³⁹

The fibrinolytic system involves a series of reactions by which plasminogen synthesized in the liver is transformed into plasmin that dissolves fibrinous networks. Homeostasis is achieved by maintaining the balance between the coagulation system and the fibrinolytic system (Fig. 5). The intensity of fibrinolysis can be evaluated by measuring the levels of FDP and d-dimer, which are decomposition products of fibrinous networks. In nasal polyps from patients with ECRS, excessive formation of fibrinous networks occurs due to increased activity of the coagulation system and the d-dimer level is low. This suggests that the fibrinolytic system is suppressed in ECRS patients. We evaluated two proteases, urokinase plasminogen activator (u-PA) and tissue plasminogen activator (t-PA), which transform plasminogen into plasmin. We found that expression of t-PA was markedly decreased in nasal polyps obtained from patients with ECRS.³⁶ Not only were t-PA mRNA and protein levels decreased, but t-PA activity was also decreased, suggesting that plasminogen activator inhibitor-1 (PAI-1; an inhibitor of both u-PA and t-PA) is also involved in the development of ECRS.

Expression of t-PA was suppressed at the DNA level. Therefore, we evaluated methylation of CpG sites at the 3'-end of the promoter region of the gene for t-PA (PLAT gene), and we showed that hypermethylation suppressed t-PA expression in nasal polyps compared with its effect in the inferior turbinate mucous membrane.⁴⁰

Nasal epithelial cells also produce t-PA. Expression of t-PA was significantly increased when cells were treated with bacterial short

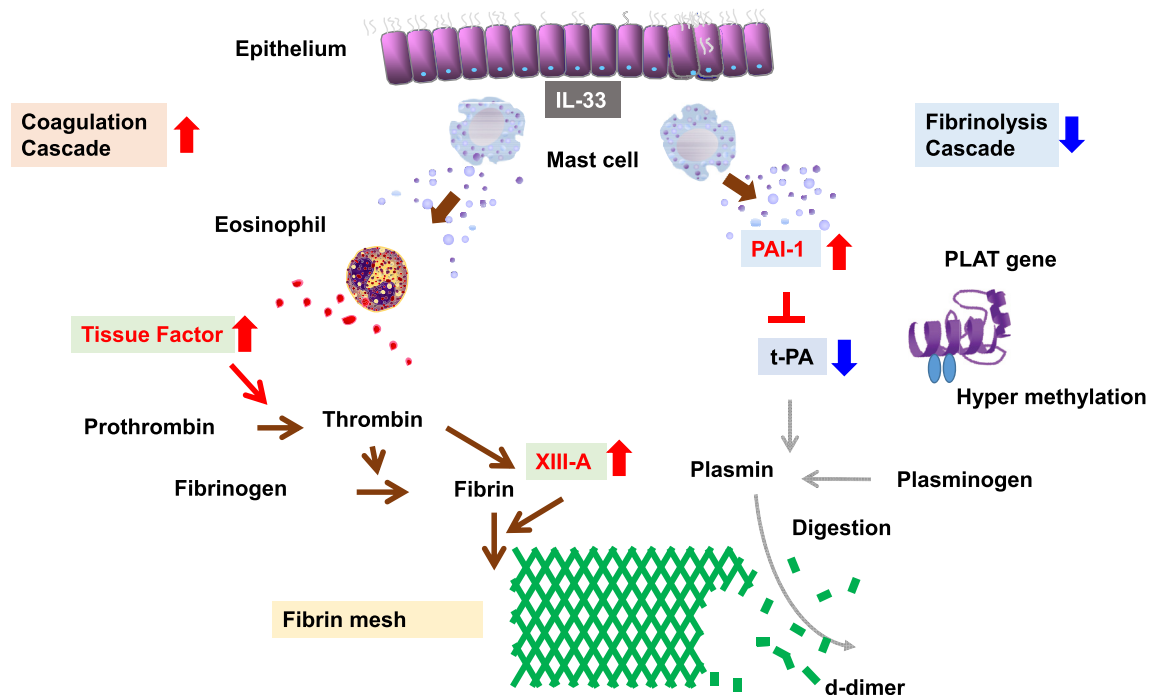


Fig. 5. Enhancement of coagulation and inhibition of fibrinolysis in ECRS.^{36–40} Coagulation cascade is activated and fibrinolysis cascade is suppressed in nasal polyps. This imbalance induces excessive formation of fibrin deposition in nasal polyps.

chain fatty acids, with this effect being mediated by expression of the short chain fatty acid receptors GPR41 and GPR43 on epithelial cells.⁴¹ This result suggests that the presence of normal nasal bacteria and/or other bacteria in the nasal cavity can help to dissolve fibrin, leading to smaller nasal polyps.

Thus, a new treatment for ECRS could involve dissolution of fibrin combined with normalization of coagulation system activity. We focused our attention on Nattokinase, a component of natto that dissolves fibrin into d-dimer. When fragments of nasal polyp tissues were treated with Nattokinase for 24 h at 37 °C, the polyp fragments became smaller and thinner. When sticky nasal discharge and sputum were treated with Nattokinase, stickiness was significantly reduced.⁴² Therefore, we conducted a large-scale multicenter placebo-controlled, randomized, comparative, double-blind study of Nattokinase in patients with ECRS. While Nattokinase was effective in some patients, the study revealed no significant differences between the Nattokinase group ($n = 101$) and the placebo group ($n = 100$) (manuscript under submission). The lack of a significant difference between the groups in our study may have arisen because the treatment period was only 8 weeks and the nasal polyp score was not evaluated uniformly.

Eosinophils and rhinosinusitis

Severe ECRS is often complicated by aspirin intolerance. Comprehensive analyses of proteins in nasal polyps from patients with aspirin intolerance and nasal polyps from patients with non-ECRS showed a significantly increase in the expression of L-plastin (LCP-1), eosinophil lysophospholipase, and Charcot-Leyden crystals (CLCs) in nasal polyps obtained from patients with aspirin intolerance.⁴³ L-plastin was mainly expressed by eosinophils. After L-plastin knockdown with siRNA in an eosinophil cell line (EoL-1 cells), cellular infiltration through GM-CSF and infiltration from vascular endothelial cells were inhibited. Additionally, L-plastin expression was associated with the expression of tissue

factor (a coagulation factor) and contributed to an increase of coagulation activity.

Furthermore, significantly more Charcot-Leyden crystals (CLCs) were observed in nasal polyps from patients with ECRS. During the process of cell death mediated by eosinophil extracellular traps (ETosis), localization and release of galectin-10 causes formation of CLCs.⁴⁴ In ECRS patients, eosinophil ETosis commences in the lamina propria of the mucous membrane, after which eosinophils migrate through the epithelium to the nasal cavity where DNA traps for bacteria exist. The damaged DNA subsequently promotes the production of viscid secretions⁴⁵ (Fig. 6), which presumably cause a viscid nasal discharge in patients with ECRS. In an *ex vivo* model of the human mucous membrane, ETosis and DNA traps were significantly correlated with an increase of *S. aureus* colony formation and increased production of IL-5 and periostin. However, *S. epidermidis* did not promote either ETosis or DNA traps.⁴⁶

Eosinophilic infiltration into the paranasal sinuses and nasal polyps may be mediated by expression of peripheral lymph node addressin (PNAd) on vascular endothelial cells. PNAd is a ligand of L-selectin. PNAd expression in the blood vessels of nasal polyps was significantly increased in ECRS patients compared with non-ECRS patients. ECRS occurs in a calcium-dependent manner, and eosinophilic infiltration was significantly inhibited when eosinophils were treated with anti-L-selectin antibody.⁴⁷

Analysis by next-generation sequencing

To find biomarkers of ECRS, we performed within-patient comparison of gene expression in nasal polyps and in the mucous membrane of the inferior turbinate by next-generation sequencing.²¹ We found significantly increased expression of 1264 genes in nasal polyp tissues, as well as significantly decreased expressions of 899 genes. Among 620 genes with an increased expression, top seven genes, *POSTN*, *CST-1*, *SERPIN3*, *CCL18*, *FDCSP*, *CXCL17*, *ALOX15* were significantly highly expressed in nasal polyps

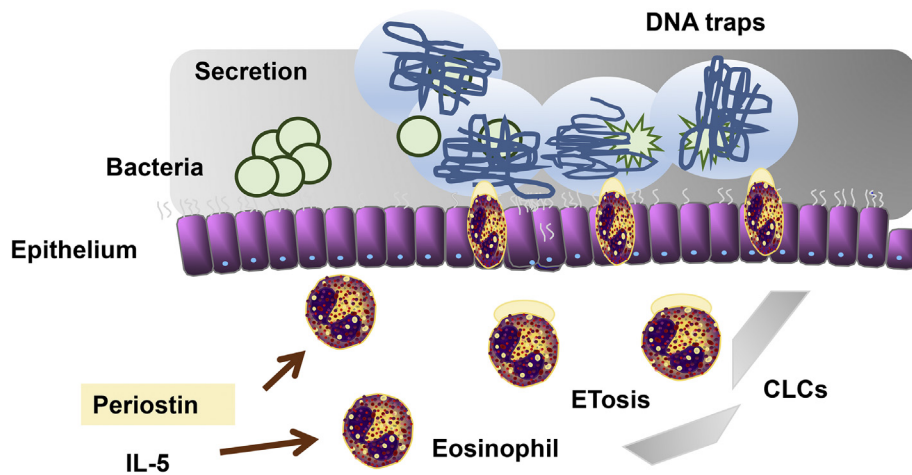


Fig. 6. ETosis and DNA traps in ECRS.^{44,45} ETosis and Charcot-Leyden crystals are observed in nasal polyps of ECRS. DNA traps for *Staphylococcus aureus* occur in CRSwNP of Europe.

than in inferior turbinate mucous membrane. The gene *POSTN* (periostin) demonstrated the highest expression in nasal polyp tissues.

Role of periostin in ECRS

We performed immunohistochemistry for periostin in nasal polyps from patients with ECRS and patients with conventional CRS. This investigation showed diffuse strong immunostaining for periostin in the nasal polyps from patients with ECRS. Then we measured the serum periostin level by ELISA (Shino-Test Corporation, Tokyo) in patients with non-ECRS, mild ECRS, moderate ECRS, and severe ECRS. We found that the serum periostin level increased significantly along with the severity of ECRS. In addition, there was a significant positive correlation between the serum periostin level and the eosinophil count in peripheral blood.²¹ Then we determined the cutoff value of periostin based on receiver operating characteristic (ROC) analysis, and we used the Kaplan Meier method to estimate the recurrence rate of nasal polyps after ESS. Our results showed that the polyp recurrence rate was significantly higher in patients with a high serum periostin level, suggesting that the preoperative serum periostin level could be a clinical marker for recurrence after ESS.

The level of periostin is correlated with those of vascular endothelial growth factor (VEGF) and regulated on activation normal T expressed and secreted (RANTES), with levels of all 3 factors being high in patients with ECRS. It was reported that the levels of VEGF and RANTES also changed as the periostin level increased or decreased *in vitro*, and another group reported that periostin is related to the onset and prognosis of ECRS.⁴⁸ Asthma is often present in patients who have CRSwNP. The serum periostin level was found to be significantly higher in patients who had asthma and CRSwNP than in patients who had asthma without nasal polyps, and the serum periostin level showed a significant positive correlation with the paranasal sinus CT score (Lund–Mackay score).⁴⁹

ECRS and protease inhibitors

After periostin, expression of CST-1 (Cystatin SN) was the next highest in nasal polyps. CST-1 is a cystatin protease inhibitor that is produced by cells in the nasal mucosa to inhibit cystatin proteases (proteolytic enzymes) from allergens, bacteria, and viruses. As was done for periostin, we performed immunohistochemical staining to detect CST-1 in nasal polyp tissues, and we found that CST-1 staining

became more intense and more diffuse along with an increase in the severity of ECRS, while CST-1 staining was weaker in non-ECRS. When the intensity of CST-1 staining was converted to a score, the score was significantly higher in ECRS than in non-ECRS, and the score showed a positive correlation with the severity of the condition. Additionally, expression of CST-1 mRNA in nasal polyps displayed a significant positive correlation with the eosinophil count in the polyps and with the level of expression of mRNAs for TSLP, IL-33, CCL11 (eotaxin-1), and periostin. Production of TSLP by nasal mucosal epithelial cells was increased in response to stimulation with IL-4, dsRNA, and CST-1, while productions of Eotaxin-1 and periostin by nasal mucosal fibroblasts increased after stimulation with CST-1.⁵⁰ Thus, CST-1 seems to be involved in the promotion of eosinophilic infiltration in patients with ECRS (Fig. 7). Subsequently, there was a report from China that CST-1 promotes the activation and migration of eosinophils,⁵¹ in support of our findings.

Cystatin A is another protease inhibitor and a member of the cystatin family. In contradiction to CST-1, cystatin A was decreased in mucous membranes from patients with ECRS. Allergens were reported to induce production of TSLP, IL-25, and IL-33 by nasal mucosal epithelial cell lines, while pretreatment with recombinant cystatin A inhibited allergen-induced production of TSLP, IL-25, and IL-33.⁵² Therefore, induction of cystatin A expression could be a new approach to the treatment of ECRS.

Serine proteases, including Spl from *S. aureus*, have been reported to act as allergens. These proteases derived from bacteria, fungi, and mites interact with each other and also interact with various inhibitory substances produced by the host, which may contribute to the pathogenesis of diseases such as AR and ECRS.⁵³

ECRS and TRPV3

We used next-generation sequencing to examine nasal polyps from patients who had ECRS and nasal polyps from patients who had non-ECRS with neutrophil-dominant inflammatory cell infiltration, revealing a difference in the expression of transient receptor potential vanilloid 3 (TRPV3).⁵⁴ TRPV3 is a Ca²⁺-permeable non-selective cation channel that is abundantly expressed on keratinocytes. It plays an important role in maintaining the barrier function of the oral epithelium and has been reported to react to thermal stimulation and to be involved in perception and transmission of heat sensation. When the expression of TRPV3 was determined by immunohistochemical analysis in patients with ECRS, it was found that TRPV3 was expressed by infiltrating cells

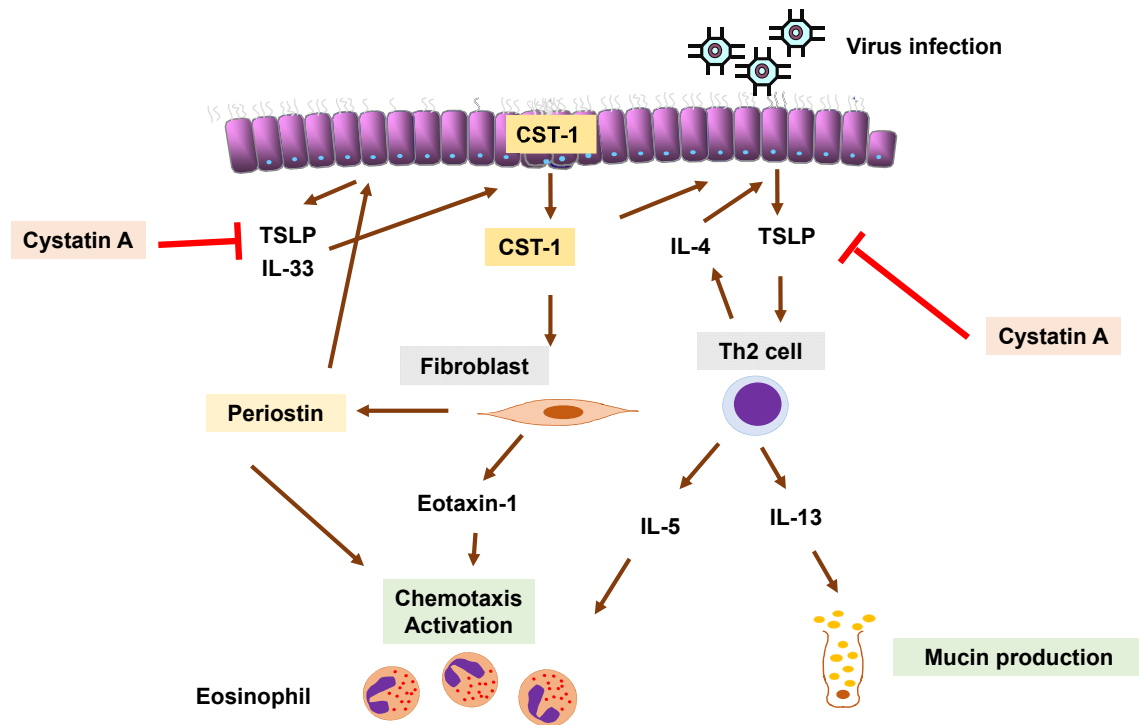


Fig. 7. Role of CST-1 and Cystatin A in ECRS.^{50,52} Virus infection and CST-1 upregulate Th2 cytokine secretion by Th2 cells and eotaxin production by fibroblast. Strong eosinophil infiltration and high mucin production are induced by CST-1. Cystatin A suppresses TSLP production by epithelial cells.

and the mucosal epithelium, with the level expression being positively correlated with the severity of the disease. TRPV3 expression also showed a positive correlation with the eosinophil count in nasal polyps, and TRPV3-positive cells displayed strong expression of ECP and were likely to be eosinophils.⁵⁴ However, the function of TRPV3 is still unknown.

Treatment for ECRS

Oral glucocorticosteroids are effective treatment to decrease nasal polyps in ECRS patients. However, oral glucocorticosteroids are not able to be administrated for a long period, because of adverse effects. Repeated short-course oral glucocorticosteroids treatments have been performed for severe ECRS patients.¹⁵ Instead of oral glucocorticosteroids, topical glucocorticosteroids are considered for the treatment of CRSwNP.¹² However, there is no evidence that topical glucocorticosteroids have been effective for ECRS in Japan. The nasal exhalation of inhaled corticosteroids decreased shadows of ethmoid sinuses of CT in ECRS patients with asthma.⁵⁵

Amoxicillin is recommended for the infectious ECRS patients. Amoxicillin has decreased purulent discharge and changed to mucous discharge. If Amoxicillin would be ineffective, antibiotics would be changed in reference of data of bacterial examination in nasal discharge. Long-term low-dose macrolide therapy has been often performed, but decrease of nasal polyps and recovery of hyposmia would not be observed.

ESS and topical glucocorticosteroid treatments after ESS are currently standard therapies for the treatment of ECRS. However, considerable numbers of patients had recurrent nasal polyps.^{10,15}

Figures 3, 4 and 7 show the mechanism of type 2 inflammation. Treatment options of nasal polyps of ECRS are to target the type 2 inflammation, which is characterized by a prominent role of cytokines, such as IL-4, IL-5, IL-13 and IgE. Clinical studies of these biologics, anti-IL-5 antibody (Ab), anti-IL-5 receptor (IL-5R) Ab, anti-

IgE Ab and anti-IL-4 α chain Ab, have been performed for severe CRSwNP. Anti-IgE Ab (omalizumab) improved nasal obstruction, hyposmia and nasal secretion scores in ECRS patients with aspirin induced asthma.⁵⁶ Placebo-controlled double-blind study of anti-IL-5 Ab (mepolizumab) demonstrated to decrease nasal polyps and to improve CT findings in patients with large nasal polyps.⁵⁷ Anti-IL-4 α chain Ab (dupilumab) improved nasal polyp score, CT score by Lund–Mackay score, QOL scores and the olfactory test score.⁵⁸ Promising results of an international phase III trial of dupilumab for CRSwNP with high polyp scores will be published soon. Anti-IL-5R Ab (benralizumab) had been effective for limited patients with ECRS. Precision medicine using biologics will be performed to ECRS patients in the future.

Conclusion

Currently, the number of patients with ECRS is increasing in Japan. Although the reason for this increase of ECRS is unknown, a change of treatment for asthma could be a possible cause. In the past, oral glucocorticosteroid were often used to treat patients with moderate or severe asthma. In 1993, the Japanese Society of Allergy published an asthma prevention and management guideline, and made efforts to encourage its acceptance. As a result, both use of oral glucocorticosteroid as treatment and deaths from asthma decreased significantly, while use of inhaled corticosteroids as an alternative treatment showed a significant increase.⁵⁹ Along with this increase in the use of inhaled corticosteroids, the number of patients with ECRS also increased. Patients with ECRS often have asthma, including aspirin-induced asthma, and only oral glucocorticosteroid are effective as treatment. Therefore, it seems reasonable to conclude that this change in the treatment of asthma may have led to the increase of ECRS. Alternatively, environmental changes may have led to alterations of the bacterial flora in the mouth, nasal cavity, and intestine, resulting in an increase of patients with ECRS. Additionally, food may be important factor for the

increase of ECRS. Fermented food, ex Natto, may regulate coagulation and fibrinolysis in nasal mucosa.

In conclusion, the etiology and pathogenesis of ECRS have still not been clarified. ECRS is rarely complicated by other diseases associated with infiltration of eosinophils, such as including eosinophilic esophagitis, eosinophilic folliculitis, or eosinophilic gastroenteritis. The mechanism underlying eosinophil infiltration in all eosinophilic diseases has not been determined, but if a common mechanism could be identified, it would lead to a breakthrough in treatment for all eosinophilic diseases.

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Conflict of interest

SF has been an advisory board member for GSK, Kyowa Hakko Kirin and Sanofi; and has received lecture fees from Kyorin, Taiho and Mitsubishi Tanabe. The rest of the authors have no conflict of interest.

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