

Original Paper

Clinical Characteristics and Expression of Thymic Stromal Lymphopoietin in Eosinophilic and Non-Eosinophilic Chronic Rhinosinusitis

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Key Words

Chronic rhinosinusitis · Eosinophils · Thymic stromal lymphopoietin · Thymic stromal lymphopoietin receptor · IL-7R

Abstract

Objective: To evaluate the differences in clinical features and expression of cytokine thymic stromal lymphopoietin (TSLP) and its receptors in patients with eosinophilic and non-eosinophilic chronic rhinosinusitis (ECRS and NECRS). **Method:** 36 ECRS patients and 50 NECRS patients were evaluated for symptoms, nasal sinus computed tomography scanning, nasal endoscopy, skin prick test (SPT) positivity, and total IgE. Expression of TSLP and receptors in ethmoid sinus mucosa from the ECRS and NECRS groups were investigated by using immunohistochemical staining. **Results:** ECRS patients demonstrated significantly higher scores of cough and hyposmia. 66.7% of ECRS patients also demonstrated nasal polyps, compared to 50% of NECRS patients, with significantly higher polyp endoscopy scores. 80.6% of ECRS patients demonstrated SPT positivity, compared to 14% of NECRS patients. The overall expression of TSLP, TSLP receptors and IL-7R was significantly greater in eosinophils in the mucosa of ECRS patients than in NECRS patients. The expression of TSLP and receptors in SPT-positive ECRS patients was significantly greater than in SPT-negative patients, with a significant correlation noted between the expression of TSLP and nasal polyp scores. **Conclusion:** The clinical manifestations of ECRS are likely to be influenced by atopic status of an individual and TSLP-mediated eosinophil infiltration of the rhinosinusoidal mucosa.

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Introduction

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinus mucosa, which can broadly be classified into two subtypes based on the presence or absence of nasal polyps [1, 2]. Evidence suggests that there is a pronounced eosinophilic infiltration of the rhinosinusoidal mucosa in some patients with CRS, classified as eosinophilic CRS (ECRS) [3], and that the level of eosinophil infiltration was associated with the severity of nasal sinusitis and a poor prognosis [4, 5]. However, the difference of clinical characteristics between Chinese ECRS and non-eosinophilic CRS (NECRS) patients remains poorly defined.

Thymic stromal lymphopoietin (TSLP), an IL-7-like cytokine produced by keratinized cells, epithelial cells, smooth muscle cells, basophils and IgE-bound mast cells [6, 7], has been identified as an important initiator of the Th2 cell response as it can activate antigen-presenting cells and dendritic cells to promote the induction of CD4+ T cells towards Th2 differentiation and development [8]. Studies investigating the expression and role of TSLP in atopic diseases like asthma and atopic dermatitis have shown elevated level of TSLP in keratinocytes of patients with allergic dermatitis [9], as well as increased TSLP expression in the bronchial mucosa and epithelial cells of patients with asthma, and in bronchoalveolar lavage fluid of patients with chronic obstructive pulmonary disease [10, 11]. Although few studies have shown TSLP expression in allergic rhinitis, to date there are no reports of the expression or role of TSLP in CRS patients.

The aim of the current study was therefore to characterize the clinical features of ECRS and NECRS in Chinese patients, and to investigate the expression of TSLP and TSLP receptors (TSLPR) in the rhinosinusoidal mucosa of patients with CRS.

Methods

Subjects

Eighty-six patients with CRS undergoing endoscopic sinus surgery in the Department of Otolaryngology, Head and Neck Surgery at Beijing Tongren Hospital from May 2009 to February 2010 were enrolled in the study. Diagnostic criteria were in accordance with the 2007 European Position Paper on Rhinosinusitis and Nasal Polyp (EPOS) [2]. Prior to surgery, all patients were assessed for symptoms and underwent sinus computed tomography (CT) examination, nasal endoscopy and allergen skin prick tests (SPT). Blood samples were also taken for assessment of serum total IgE (normal range: 0–60 kU/l, ALK). None of the patients had previous history of sinus surgery and patients with antrochoanal polyps, cystic fibrosis, fungal sinusitis, primary ciliary dyskinesia, or gastroesophageal reflux disease were excluded from the study. The study was approved by the ethics committee of the Capital Medical University, and all patients gave written informed consent to participate in the study.

Hypertrophied and hyperemic ethmoid sinus mucosa tissue samples were collected during surgery, and one half of each sample was immediately snap-frozen in liquid nitrogen and stored at -80°C for RNA extraction. The other half of each sample was fixed in 4% formaldehyde solution and processed for hematoxylin and eosin (HE) and immunohistochemical staining.

Based on the predominant types of inflammatory cells seen in the mucosal samples, patients were classified as either ECRS or NECRS subjects; with ECRS subjects demonstrating numerous eosinophils and expression of EG2 and NECRS subjects demonstrating greater numbers of neutrophils and monocytes. Based on this classification there were 36 patients in the ECRS group (22 males, 14 females; aged 14–68 years, mean age 44.6 ± 14.9 years), and 50 patients in the NECRS group (31 males, 19 females; aged 14–70 years, mean age 40.2 ± 16.3 years).

Assessment of Clinical Characteristics

Symptom Score

Symptom assessment was based on the sino-nasal outcome test-20 (SNOT-20) score [12], including the severity of nasal obstruction, nasal discharge, head and facial pain or pressure, decreased sense of smell, ear

pressure or pain, cough, and tear reflux. A score of 0 indicated that the patient was unaffected, a score of 1 = slightly affected, a score of 2 = moderately affected, and a score of 3 = seriously affected.

Nasal Sinus CT Scan Score

Preoperative three-dimensional CT scan was performed on the nasal sinuses. Quantitative assessment of CT scan results was based on the Lund-Mackay score system according to the following scale [13]: 0 = normal sinuses and no opacification, 1 = partial opacification, and 2 = complete opacification. The osteomeatal complex was scored as 0 (not occluded) or 2 (occluded).

Nasal Endoscopy Score

Quantitative assessment by nasal endoscopy examination utilized the Lanza-Kennedy scoring system [14] according to the following scale for edema: 0 = no edema in the bilateral nasal mucosa, 1 = mild edema, and 2 = severe edema. Similarly, polyps were scored as 0 = no nasal polyps, 1 = polyps within the middle nasal meatus, and 2 = polyps beyond the middle nasal meatus.

Immunohistochemical Analysis

Samples fixed in 4% formaldehyde solution were embedded in paraffin and sliced according to conventional histologic techniques. Sections were incubated with anti-human EG2 standardized at a 1:150 dilution (Pharmacia), anti-TSLP (1:200), anti-TSLPR (1:200), anti-IL-7R α (1:1,000) (R&D Systems, Minneapolis, Minn., USA), or IgG antibody (negative control, Santa Cruz Biotechnology, Inc.). Immunostaining was carried out using a standard Vecta stain Avidin-Biotin complex kit (Vector Laboratories), and color development was achieved with 3'-3'-diaminobenzidine (DAB). Five randomly selected high power fields ($\times 400$ magnification) were assessed by two independent investigators blinded to clinical status of the subjects and the results were expressed as mean number of positively-stained cells/field. The sections were photographed with a digital color-CCD camera (Olympus BX50, Tokyo, Japan).

RT-PCR

Total RNA was extracted from the samples stored in liquid nitrogen, using the RNeasy Mini Kit (Qiagen, Valencia, Calif., USA). After quantification of nucleic acids, 1 μ g mRNA from each sample was reverse-transcribed into cDNA using the Revert Aid 1st Strand cDNA Synthesis Kit (Fermentas, Burlington, Ont., Canada). Primer sequences for TSLP, TSLPR, IL-7R and β -actin used for the PCR reactions were as follows: TSLP forward, 5'-GGGCTGGTGTTAACTTACGACT-3', reverse, 5'-ATTGCTACAAGAGACGGTGTG-3'; TSLPR forward, 5'-TGAGATTTTCGTGGCATCAG-3', reverse, 5'-GACCAGTCGCTTGGGTATG-3'; IL-7R α forward, 5'-GAAGGTTG-GAGAAAAGAGTC-3', reverse, 5'-CAAAATGCTGATGGTTAGT AAG-3'; and β -actin forward, 5'-CTTCCTTC CTGGGCATGGAG-3', reverse, 5'-TGGAGGGGCCGACTCGTCA-3' (Sangon, Shanghai, China). The PCR reaction protocol comprised 32 cycles of incubations at 93°C for 3 minutes, 95°C for 30 s, 60°C for 30 s (1 minute for TSLPR), and 72°C for 1 min, followed by a final extension at 72°C for 10 minutes. PCR products were analyzed by agarose gel electrophoresis and ABB software was used to analyze the mRNA expression of TSLP, TSLPR and IL-7R. The ratio of the grey values of TSLP, TSLPR or IL-7R electrophoresis bands to the grey value of β -actin was considered to be the corresponding relative amount.

Statistical Analysis

SPSS v13.0 package was used for statistical analysis. ANOVA analysis was performed to compare the mean between groups and the correlation between two variables was analyzed using the Spearman method. p values <0.05 were considered statistically significant.

Results

Patient Characteristics

Figure 1 shows the subjective symptom scores for all patients. Of the seven main symptoms documented, olfactory disorders and cough scores were significantly higher in ECRS patients than in NECRS patients ($p < 0.05$). The two groups of patients were not significantly different with respect to the symptoms of nasal obstruction, nasal discharge, tear reflux, ear pressure or pain, or facial pain or pressure.

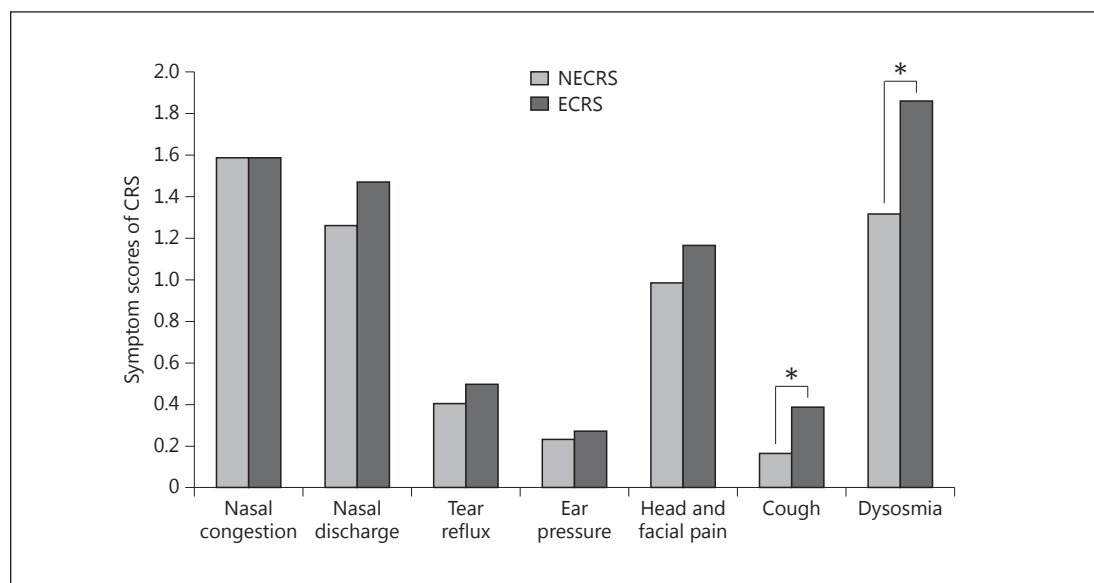


Fig. 1. Subjective symptom scores in ECRS and NECRS patients. Symptoms of dysosmia and cough in ECRS patients scored significantly higher than in NECRS patients (* $p < 0.05$); with no significant differences for the symptoms of nasal congestion, nasal discharge, tear reflux, ear pressure or pain, or head and facial pain or pressure between the two groups.

Table 1. Clinical characteristics of ECRS and NECRS patients

	Case number	CT score	Score of nasal polyp degree	Concurrent rates of nasal polyps, %	SPT-positive rate, %	Average IgE
ECRS	36	12.36±6.85	1.94±1.66*	66.7 (24/36)	80.6 (29/36)**	120.3±151.14
NECRS	50	10.86±6.81	1.38±1.53	50.0 (25/50)	14 (7/50)	48.0±65.29

* $p < 0.05$, ** $p < 0.01$.

Analysis of objective clinical examinations carried out is shown in table 1. Nasal polyps occurred in 66.7% (24/36) of ECRS patients and 50% (25/50) of NECRS patients. Nasal polyp severity (score of nasal polyp degree) was significantly higher ($p < 0.05$) in the ECRS group. CT examination indicated no significant differences between the two groups. SPT-positive rates for ECRS group and NECRS group were 80.6% (29/36) and 14% (7/50), respectively. Although mean total serum IgE levels also differed greatly between the two groups (120.3 kU/l in ECRS group and 48.0 kU/l in NECRS group), the difference was not found to be statistically significant.

Expression of TSLP and Receptors in ECRS and NECRS Groups

Immunohistochemical staining showed that TSLP, TSLPR, and IL-7R α were expressed in epithelial cells, plasma cells and fibroblasts in both groups. Most importantly, TSLP and its receptors were also significantly expressed in eosinophils in the ECRS group (fig. 2). Consistent with these results, mRNA levels of TSLP and its receptors in ECRS group ($n = 14$) were found

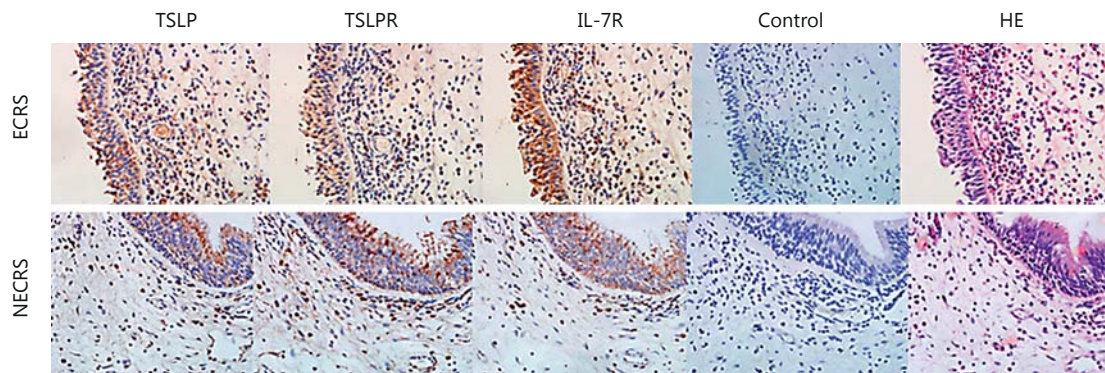


Fig. 2. Expression of TSLP and receptors in the sinus mucosa of ECRS and NECRS patients. Immunohistochemical staining indicated that TSLP, TSLPR and IL-7R α were mainly expressed in eosinophils, epithelial cells, plasma cells and fibroblast cells in both groups. In the ECRS group, TSLP, TSLPR and IL-7R α were significantly expressed in eosinophils ($\times 400$ magnification).

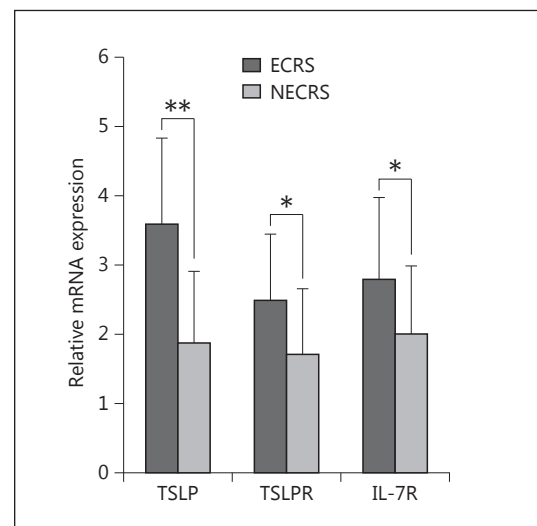


Fig. 3. mRNA level of TSLP and receptors in sinus mucosa in ECRS and NECRS patients. RT-PCR results indicated that mRNA expression levels of TSLP, TSLPR and IL-7R α were all higher in the ECRS group ($n = 14$) than in the NECRS group ($n = 11$) (intergroup comparison: ** $p < 0.01$ for TSLP; * $p < 0.05$ for TSLPR and IL-7R).

to be higher than in NECRS group ($n = 11$) ($p < 0.01$ for TSLP; $p < 0.05$ for TSLPR and IL-7R, fig. 3).

Expression of TSLP and Receptors in SPT-Positive and -Negative Patients in ECRS Group

Assessment of the expression of TSLP and its receptors in ECRS patients demonstrated that this was significantly higher in SPT-positive patients ($n = 7$) than in SPT-negative patients ($n = 7$), as indicated by both immunohistochemical staining (fig. 4a) and mRNA levels (fig. 4b) ($p < 0.01$ for both TSLP and TSLPR; $p < 0.05$ for IL-7R).

Correlation between TSLP Expression, Nasal Polyp Severity and Total Serum IgE Levels

In ECRS group, TSLP expression was correlated with nasal polyp severity ($r = 0.735$); however, the correlation between mRNA levels of TSLP and total serum IgE levels was poor ($r = 0.510$, $p > 0.05$) (fig. 5).

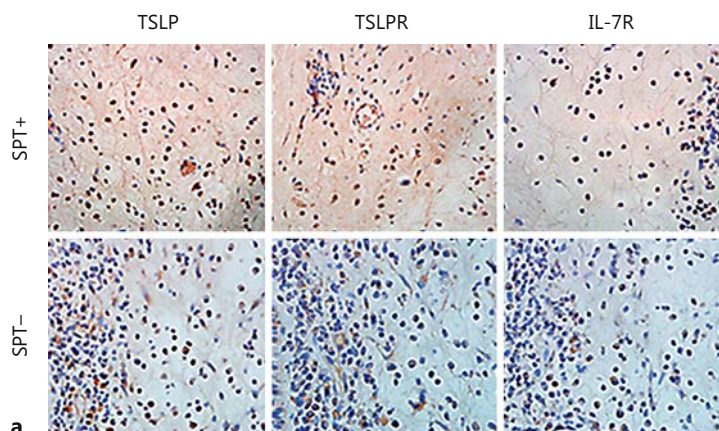


Fig. 4. Expression of TSLP and receptors in SPT-positive and SPT-negative patients. Expression of TSLP, TSLPR and IL-7R α in the sinus mucosa of SPT-positive patients ($n = 7$) were all higher than in SPT-negative patients ($n = 7$) in the ECRS group. Immunostaining results (**a**) and RT-PCR results (**b**) (intergroup comparison: ** $p < 0.01$ for TSLP and TSLPR; * $p < 0.05$ for IL-7R).

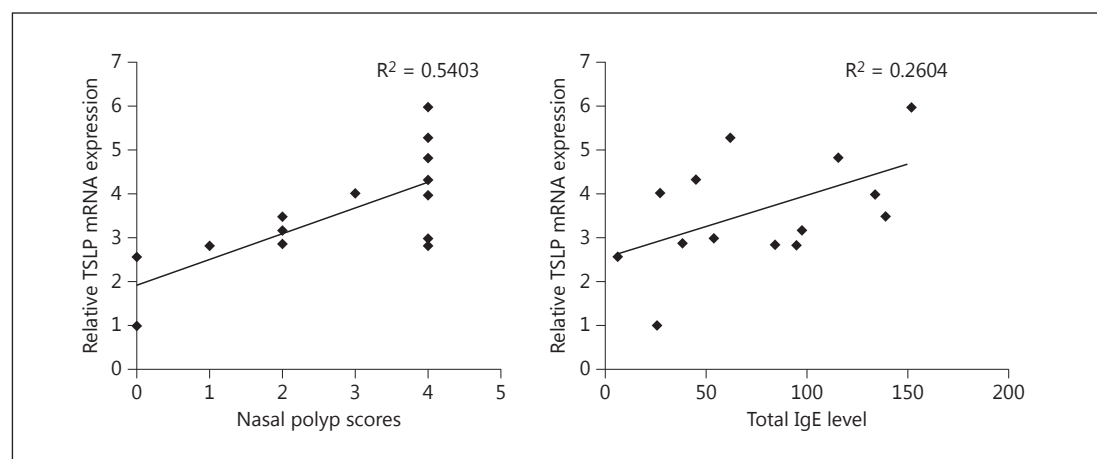
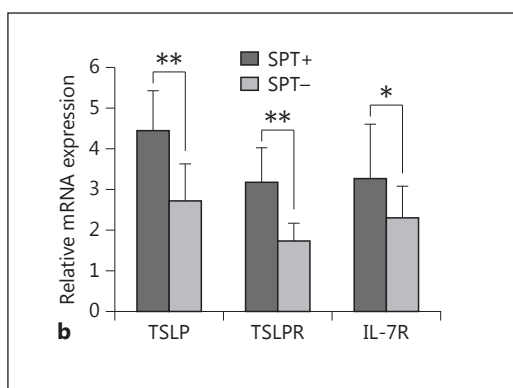


Fig. 5. Correlation between TSLP expression and severity of nasal polyps. In the ECRS group, expression of TSLP was significantly correlated to nasal polyp severity ($r = 0.735$, $p < 0.01$), whereas the correlation between expression of TSLP and total serum IgE levels was weak ($r = 0.510$, $p > 0.05$).

Discussion

ECRS is an inflammatory disease characterized by increased numbers of eosinophils in particular, as well fibroblasts, mast cells, goblet cells, and Th2 lymphocytes. Although the underlying cause of ECRS is not clear, bacteria and fungi may act as immune stimulators or as superantigens. Clinical studies have also confirmed that some patients with ECRS cannot be controlled by endoscopic sinus surgery (ESS) and macrolide therapy, and there is a tendency for recurrence of the disease after surgery and/or systemic steroid therapy [15, 16]. Thus, a distinction between ECRS and NECRS before surgery may be an effective therapeutic strategy for the management of CRS patients.

Our study has demonstrated that there is a difference in the symptom scores between the ECRS and NECRS patients, with particularly cough and dysosmia being significantly higher in ECRS patients than in NECRS patients and that these symptoms were correlated with infiltration of a large number of eosinophils into the tissue. There is evidence that activated eosinophils in the bronchial mucosa of patients with bronchial asthma was a major cause of coughing [17, 18], whereas severe olfactory epithelial edema and inflammation are the main causes for dysosmia in CRS patients [19]. In the inflammatory process, activated eosinophils can release chemokines (such as RANTES, eotaxin, and platelet-activating factor) and the lipid mediators such as leukotrienes, which can increase vascular permeability, mucosal edema, and mucus secretion. In addition, eosinophils can also enhance the synthesis and effects of bioactive substances like cytokines (IL-3, IL-5 and GM-CSF), leading to respiratory mucosa damage, severe mucosal inflammation and edema, coughing, olfactory dysfunction and the development of polyp.

Studies from the US and Europe have suggested that eosinophil infiltration in CRS patients with nasal polyps was significantly higher than in CRS patients without nasal polyps [20, 21]. Although we were unable to show any significant difference in the proportion of patients with concurrent nasal polyps between the two groups (66.7% for the ECRS group and 50% for the NECRS group), our study demonstrated that the severity scores of nasal polyps in the ECRS group were significantly higher than in the NECRS group. Furthermore, our study demonstrated that nearly six times as many patients in the ECRS group (80.6%) were SPT-positive, compared to only 14% of the patients in the NECRS group; and total IgE in ECRS was higher than in NECRS group, suggesting that allergic factors play an important role in the inflammation process in ECRS. Increased levels of IgE may be due to abnormal regulation of IgE production caused by superantigens from bacteria. Bacterial enterotoxins can stimulate IgE production by targeting T and B cell receptors and major histocompatibility complex. Indeed, some studies have demonstrated that 25–58% of individuals with sinusitis have allergic rhinitis and are sensitized especially to perennial allergens [22, 23]. Moreover, some authors argue that ECRS and asthma share similar histopathologic features and are the same inflammatory process manifested in different sites [24].

Kimura et al. [25] found high expression of TSLP in nasal polyps in patients with atopy, which is positively related to the number of eosinophils in the tissue. Similarly, Wong et al. [26] have demonstrated that TSLPR was expressed in eosinophils isolated and cultured from the blood of healthy volunteers. Consistent with these findings, our study demonstrated that TSLP was expressed in epithelial cells, plasma cells and inflammatory cells in the nasal mucosa of patients in both the ECRS and NECRS groups. Moreover, for the first time, our study has demonstrated that TSLP and its receptors (TSLPR and IL-7R) were expressed at significantly higher levels by eosinophils in patients with ECRS compared to patients with NECRS. The expression of TSLP and its receptors was also found to be significantly higher in the SPT-positive group than in the SPT-negative group and the expression of TSLP mRNA was positively related to the severity of nasal polyps. Collectively, these findings suggest that TSLP and

its receptors are closely associated with eosinophil-dominated allergic inflammation and that epithelial derived TSLP may bind TSLP receptors on the eosinophil surface to activate eosinophils participating in the Th2 immune response and induction of mucosal inflammation and edema noted in CRS patients. Some studies have also suggested that there may also be an association between TSLP expression and allergic inflammation in allergic dermatitis and asthma. One study showed that TSLP was highly expressed in keratinocytes in the inflammation skin zone in patients with allergic dermatitis, but not in normal keratinocytes [9]. Similarly, other studies have shown TSLP expression to be significantly higher in bronchial epithelial cells in patients with asthma, compared with bronchial epithelial cells in normal controls [10, 11]. Although the findings of the present study suggest a similar role of TSLP in sinus mucosa of ECRS patients as that in other allergic inflammatory conditions, the specific mechanisms underlying TSLP-mediated activation of eosinophils in these conditions need to be elucidated further.

In conclusion, our study has demonstrated that patients with ECRS experience significantly more severe symptoms of olfactory dysfunction and cough, nasal polyp severity and positive allergic reactions compared to patients with NECRS. These clinical manifestations are closely related to the characteristic eosinophilic infiltration in ECRS, which may in turn be a consequence of significantly greater expression of TSLP and its receptors than in the NECRS patients.

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