# Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis

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**Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is associated with $T_H2$-dominant inflammation. Thymic stromal lymphopoietin (TSLP) is a cytokine that triggers dendritic cell–mediated $T_H2$ inflammatory responses and that enhances IL-1–dependent $T_H2$ cytokine production in mast cells. Although increased TSLP mRNA levels have been found in nasal polyps (NPs), expression of TSLP protein and its function in patients with chronic rhinosinusitis (CRS) have not been fully explored.**

**Objectives: The objective of this study was to investigate the role of TSLP in patients with CRS.**

**Methods: We investigated the presence and stability of TSLP protein in NPs using ELISA and Western blotting and investigated the function of TSLP in nasal tissue extracts with a bioassay based on activation of human mast cells.**

**Results: Although TSLP mRNA levels were significantly increased in NP tissue from patients with CRSwNP compared with uncinate tissue from patients with CRS or control subjects, TSLP protein was significantly decreased in NP tissue, as detected by using the commercial ELISA kit. We found that recombinant TSLP was time-dependently degraded by NP extracts, and this degradation was completely inhibited by a protease inhibitor cocktail, suggesting that TSLP is sensitive to tissue proteases. Interestingly, NP extract–treated TSLP had higher activity in mast cells, although the amount of full-length TSLP was reduced up to 85%. NP extracts significantly enhanced IL-1β–dependent IL-5 production in mast cells compared with uncinate tissue homogenates, and responses were significantly inhibited by anti-TSLP, suggesting that NPs contain biologically relevant levels of TSLP activity.**

**Conclusion: TSLP and its metabolic products might play an important role in the inflammation seen in patients with CRSwNP. (J Allergy Clin Immunol 2013;132:593-600.)**

***Key words:*** *Chronic rhinosinusitis, nasal polyps, thymic stromal lymphopoietin, epithelial cells, mast cells, $T_H2$ cells, IL-5, proteases*

Chronic rhinosinusitis (CRS) is a heterogeneous disease characterized by local inflammation of the upper airways that persists for at least 12 weeks. CRS is one of the most common chronic diseases in adults in the United States, affecting more than 10 million Americans, and has a severe effect on patients' quality of life.[1-4] CRS is frequently divided into 2 groups based on histology and physical examination: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). In general, CRSwNP is associated more closely with clinical complaints of nasal obstruction and olfactory loss and is characterized by eosinophilia and $T_H2$-related inflammation, especially in western countries.[5,6] However, the mechanisms underlying the amplification of $T_H2$-related inflammation in patients with CRSwNP have not been identified.

Thymic stromal lymphopoietin (TSLP), an epithelial cell–derived cytokine, is now widely recognized as a master regulator of $T_H2$ inflammation. TSLP is an IL-7–like cytokine that stimulates dendritic cells (DCs) to induce naive $CD4^+$ T-cell differentiation into $T_H2$ cells.[7-13] Although TSLP alone is not sufficient to stimulate mast cells, TSLP synergizes with the inflammatory cytokine IL-1 to potently activate mast cells to produce $T_H2$ cytokines, including IL-5 and IL-13.[14,15] TSLP, which is produced mainly by epithelial cells, is also known to be produced by skin keratinocytes, stromal cells, smooth muscle cells, fibroblasts, and mast cells.[7,9,13,16] The thymic stromal lymphopoietin receptor (TSLPR) is a heterodimeric receptor consisting of the IL-7 receptor α chain and a common γ-like TSLPR.[11-13] Considerable evidence now implicates TSLP in the pathogenesis of several $T_H2$-related inflammatory diseases, including atopic dermatitis, bronchial asthma, and eosinophilic esophagitis.[9,17-20]

Although CRSwNP is a $T_H2$-related disease, there are limited studies investigating the role of TSLP in patients with CRS and in nasal polyps (NPs) in particular.[21,22] In this study we investigated the presence and activity of TSLP in patients with CRS and found that TSLP activity was increased in NPs. Additionally,

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*Abbreviations used*
CRS: Chronic rhinosinusitis
CRSsNP: Chronic rhinosinusitis without nasal polyps
CRSwNP: Chronic rhinosinusitis with nasal polyps
DC: Dendritic cell
dsRNA: Double-stranded RNA
ILC2: Type 2 innate lymphoid cell
IMDM: Iscove modified Dulbecco medium
mDC: Myeloid dendritic cell
2-ME: 2-Mercaptoethanol
NHBE: Normal human bronchial epithelial cell
NMFF: Northwestern Medical Faculty Foundation
NP: Nasal polyp
PIC: Protease inhibitor cocktail
PNEC: Primary nasal epithelial cell
SCF: Stem cell factor
TLR: Toll-like receptor
TSLP: Thymic stromal lymphopoietin
TSLPR: Thymic stromal lymphopoietin receptor
UT: Uncinate tissue

we found that TSLP was processed by endogenous proteases in tissue, which can convert it to a more active form.

## METHODS

### Patients and biopsy specimens

Patients with CRS were recruited from the Allergy-Immunology Clinic and the Otolaryngology Clinic of the Northwestern Medical Faculty Foundation (NMFF) and the Northwestern Sinus Center at NMFF. Sinonasal and NP tissues were obtained from routine functional endoscopic sinus surgery in patients with CRS. All subjects met the criteria for CRS, as defined by the American Academy of Otolaryngology–Head and Neck Surgery Chronic Rhinosinusitis Task Force.[2] Patients with an established immunodeficiency, pregnancy, coagulation disorder, Churg-Strauss syndrome, or diagnosis of classic allergic fungal sinusitis or cystic fibrosis were excluded from the study. Details of subjects' characteristics are included in Table I and in the Methods section in this article's Online Repository at www.jacionline.org. All subjects signed informed consent forms, and the protocol governing procedures for this study was approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine.

### Cell culture

Human primary nasal epithelial cells (PNECs) were collected from the uncinate tissue (UT) or NP tissue by means of curettage with a Rhinoprobe (Arlington Scientific, Springville, Utah), as described previously.[23] Human peripheral blood–derived mast cells were obtained, as described previously.[24] Recombinant TSLP was preincubated with BSA (control) or NP extracts for 24 hours, and then mast cells were stimulated with those mixtures in the presence of 20 ng/mL IL-1β for 48 hours. Further details can be found in the Methods section in this article's Online Repository.

### Real-time PCR

Real-time RT-PCR was performed with the TaqMan method, as described previously.[25] Primer and probe sets were purchased or synthesized from Applied Biosystems (Foster City, Calif). The mRNA expression levels were normalized to the median expression of the housekeeping genes β-glucuronidase *(in vivo)* and β-actin (*in vitro* experiments). Details can be found in the Methods section in this article's Online Repository.

### ELISA, cytometric bead array, and Western blotting

The concentration of TSLP in cell-free supernatants was determined by using a commercial ELISA kit (R&D Systems, Minneapolis, Minn). The minimal detection limit for this kit is 15.6 pg/mL. The concentration of TSLP in tissue homogenates was normalized to the concentration of total protein, as detected with the BCA protein assay kit (Thermo Scientific, Rockford, Ill). The concentration of IL-5 in cell-free supernatants was measured with a cytometric bead array flex set from BD Biosciences (San Jose, Calif). The limit of detection is 2.5 pg/mL. Western blot analysis was performed with 50 ng/mL biotinylated goat anti-human TSLP antibody (R&D Systems). Further details can be found in the Methods section in this article's Online Repository.

### Statistics

All data are reported as medians (25% to 75% interquartile ranges) or as means ± SEMs. Differences between groups were analyzed by using 1-way ANOVA or the paired Student *t* test. Correlations were assessed by using Spearman rank correlation. A $P$ value of less than .05 was considered significant.

## RESULTS

### TSLP expression in patients with CRS

To examine whether nasal epithelial cells can produce TSLP, we collected epithelial scrapings and cultured PNECs. We found that double-stranded RNA (dsRNA) strongly induced the production of TSLP (73.8 ± 12.8 pg/mL, n = 5; see Fig E1, *B*, in this article's Online Repository at www.jacionline.org) and that IL-4 synergistically enhanced dsRNA-dependent TSLP production (149.7 ± 10.8 pg/mL, n = 3; see Fig E1, *B*) in PNECs (details can be found in the Results section in this article's Online Repository at www.jacionline.org). This suggests that nasal epithelial cells produce TSLP.

Because we found evidence that the resident epithelial cells in nasal mucosa can produce TSLP, we examined the relevance of TSLP in patients with CRS. To measure the expression of TSLP in patients with CRS, UTs, NP tissues, and epithelial scrapings were collected from patients with CRSsNP and patients with CRSwNP, as well as from control subjects. We found that TSLP mRNA levels were significantly increased in epithelial scrapings from NPs compared with UT from control subjects and patients with CRSsNP (Fig 1, *A*). We also examined the expression of TSLP and TSLPR in whole sinus tissue and found that mRNAs for TSLP and TSLPR were significantly increased in NP tissue from patients with CRSwNP in comparison with UT from either patients with CRS or control subjects (Fig 1, *B* and *C*). Interestingly, TSLP expression positively correlated with TSLPR levels in sinus tissue (Fig 1, *D*). However, we found no significant difference in TSLP levels between atopic and nonatopic patients or asthmatic and nonasthmatic patients (see Fig E2 in this article's Online Repository at www.jacionline.org). To examine this observation at the protein level, we prepared detergent extracts of UT and NP tissues and measured the concentration of TSLP using ELISA. Surprisingly, TSLP protein levels were significantly decreased in NP tissue from patients with CRSwNP (n = 35, 27.8 ± 6.4 pg/mg) compared with UT from patients with CRSsNP ($P < .001$, n = 25, 51.2 ± 6.2 pg/mg), patients with CRSwNP ($P < .001$, n = 24, 41.9 ± 5.0 pg/mg), and control subjects ($P < .001$, n = 17, 153.2 ± 31.0 pg/mg; Fig 1, *E*). Levels of TSLP protein in control UT were higher than in any other tissue set ($P < .05$).

### Stability of TSLP in NPs

It is known that TSLP is susceptible to mast cell proteases and that mast cell numbers are increased in NPs.[26,27] In