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Increased Expression and Role of Thymic Stromal Lymphopoietin in Nasal Polyposis

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Purpose: Nasal polyposis is a chronic inflammatory disease of the upper airways often associated with asthma and characterized by markedly increased numbers of eosinophils, Th2 type lymphocytes, fibroblasts, goblet cells and mast cells. Previous studies have shown elevated levels of thymic stromal lymphopoietin (TSLP) in atopic diseases like asthma, atopic dermatitis and mainly in animal models of allergic rhinitis (AR). Here, we investigated the expression of TSLP in nasal polyps from atopics and non-atopics in comparison with the nasal mucosa and its potential role in nasal polyposis. **Methods:** Messenger RNA expression for TSLP, thymus and activation-regulated chemokine (TARC) and macrophage derived chemokine (MDC) in nasal polyps and nasal mucosa of atopics and non-atopics was analyzed by real time PCR. Immunoreactivity for TSLP in nasal polyps and in the nasal mucosa of patients with AR and non-allergic rhinitis (NAR) was analyzed by immunohistochemistry. Eosinophil counts was analyzed by Wright-Giemsa staining and nasal polyp tissue IgE, by ELISA. **Results:** Messenger RNA expression for TSLP,TARC and MDC was markedly higher in nasal polyps as compared to the allergic nasal mucosa. Immunoreactivity for TSLP was detected in epithelial cells, endothelial cells, fibroblasts and inflammatory cells of the nasal mucosa and nasal polyps. The number of TSLP+ cells was significantly greater in the nasal mucosa of AR than NAR patients. The number of TSLP+ cells in nasal polyps from atopics was significantly greater than that of non-atopics and that in the allergic nasal mucosa. The number of TSLP+ cells correlated well with the number of eosinophils and the levels of IgE in nasal polyps. **Conclusions:** The high expression of TSLP in nasal polyps and its strong correlation to eosinophils and IgE suggest a potential role for TSLP in the pathogenesis of nasal polyps by regulating the Th2 type and eosinophilic inflammation.

Key Words: Nasal polyps; Th2 cytokines; TSLP; eosinophils; IgE

INTRODUCTION

Nasal polyposis is a chronic inflammatory disease of the upper airways often associated with asthma. Although the precise mechanisms underlying the genesis and development of nasal polyposis are not fully well defined, nasal polyps are characterized by markedly increased numbers of eosinophils, T-helper type 2 (Th2) type lymphocytes, fibroblasts, goblet cells and mast cells.[1-4] Recent studies have focused on identifying the mechanisms of eosinophil chemotaxis, activation, and survival, in addition to their possible link to gross histopathologic changes such as pseudocyst formation. Interleukin (IL)-5, transforming growth factor-beta, RANTES and eotaxin seem to be crucial players in the regulation of eosinophilic inflammation and extracellular matrix breakdown. Studies have also demonstrated a strong local upregulation of IL-13, thymus and activation-regulated chemokine (TARC), immunoglobulin E (IgE) synthesis with the formation of specific IgE to Staphylococcus aureus enterotoxins, suggesting a possible role of superantigens in these pathologic processes.[5-7]

Thymic stromal lymphopoietin (TSLP) is an IL-7 like cytokine molecule that binds to TSLP receptor (TSLPR) consisting of the IL-7 receptor α-chain (IL-7Rα) and a common γ receptor-like chain (TSLP-γ).[8-10] As the name suggests, it was originally isolated from a mouse thymic stromal cell line and characterized as a lymphocyte growth factor.[11] TSLP is produced by several cell types, including human epithelial cells and stromal and muscular cells.[12,13] It was first identified as a growth factor for B cells in a thymic stromal cell line. This cytokine presents a structural

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and functional homology to IL-7.[9] TSLPR is a heterodimer consisting of a specific TSLPR and IL-7Rα. By interacting with the heterodimeric receptor IL-7Rα/TSLPR, TSLP appears to initiate signal transduction through signal transducer and activator of transcription (STAT) 3 and STAT5 activation.[10,14-16]

It is established that Th2 cytokines IL-4, IL-5, and IL-13 play essential roles in the development of allergic disease.[7,17,18] TSLP stimulates human myeloid dendritic cells (DCs) which uniquely express the heterodimeric TSLP receptor to promote Th2 polarization of inflammatory CD4+ T cells that primes CD4+ T cells to undergoing Th2 differentiation.[15] In fact, TSLP instructs dendritic cells to induce a Th2 response. These TSLP-DC stimulate CD4(+)T cells to induce a proallergic cytokine profile, indicating an important role for TSLP plays in allergic diseases.[15]

Recently, the expression and role of TSLP in atopic diseases like asthma and atopic dermatitis were investigated and the elevated levels of TSLP in these atopic diseases and the specific expression in epithelial cells suggest its ability to trigger dendritic cell-mediated activation of Th2 inflammatory responses.[19] Although few studies have shown TSLP expression in the nasal mucosa[20] especially nasal epithelial cells and in the animal allergic rhinitis (AR) model,[21] there are no studies describing the expression or role of TSLP in nasal polyps. Furthermore, although eosinophilic nasal polyps is characterized by Th2 inflammation, the mechanism underlying the predominance of Th2 responses still needs to be clarified. Recently, we reported that nasal polyp fibroblasts in vitro can produce TSLP in response to stimulation by tumor necrosis factor (TNF) alpha and that combined stimulation with TNF-alpha + a Th2 cytokine (IL-4 orIL-13) enhanced the production of TSLP from nasal polyp fibroblasts.[5,6] In the present study, we investigated the expression of TSLP in nasal polyps from atopic as well as non-atopics in comparison with that in the nasal mucosa of patients with allergic and non-allergic rhinitis, by immunohistochemistry and its relation to eosinophil inflammation and IgE.

The airway is often exposed to not only Th2 cytokines but also bacterial products such as lipopolysaccharides (LPS). A CC chemokine, TARC, is a potent chemoattractant for Th2 cells. We recently demonstrated increased IL-4 and LPS induced TARC production by nasal polyp fibroblasts[5,22] that may play an important role in the recruitment of Th2 cells into the sinus mucosa as well as nasal polyps. We therefore also examined the mRNA expression of TSLP, TARC, and macrophage derived chemokine (MDC) in nasal polyps from atopics versus non-atopics and nasal mucosa from patients with AR.

MATERIALS AND METHODS

Patient profile

Twenty patients with nasal polyposis were included in this study. Of the twenty patients with nasal polyposis one patient had aspirin induced asthma. Ten of the twenty patients with nasal polyposis were atopic (M:F=8:2; mean age, 42.9±14.5 years) and 10 were non-atopic (M:F=10:0; mean age, 45.8±18.3 years). All the ten atopic patients with nasal polyposis had typical clinical symptoms of nasal allergy comprising sneezing, rhinorrhea and nasal congestion and positive serum specific IgE as analyzed by radioallergosorbent test (RAST). Of the 10 atopic patients with nasal polyposis and nasal allergy, 6 had perennial allergic rhinitis (PAR) to house dust (HD) mite (RAST ranged from 0.84-64.25 IU/mL), 4 had seasonal allergic rhinitis (SAR) to Japanese cedar pollen (RAST ranged from 0.48-59.12 IU/mL) and 1 had serum specific IgE to both HD mite and Japanese cedar pollen and 1 had positive serum specific IgE to dog (RAST 0.43IU/mL). In this study, we also included ten patients with AR (M:F=8:2; mean age, 40.4±13.5 years), and 10 with non-allergic rhinitis (NAR) (M:F=9:1; mean age, 35±12.25 years).

The diagnosis of AR was done on the basis of a typical history of clinical symptoms of sneezing, rhinorrhea and nasal congestion, clinical examination by anterior rhinoscopy and positive serum specific IgE by RAST. All the 10 patients with AR had PAR to HD mite (RAST ranged from 0.52 to 58.50 IU/mL), seven of them also had SAR to Japanese cedar pollen (RAST ranged from 0.57 to 38.10 IU/mL). Patients with NAR had hypertrophied turbinates but with no symptoms of nasal allergy. All patients were symptomatic at the time of collecting the specimens and none of them had received immunotherapy in the past. Nasal polyp specimens and nasal mucosal tissue samples were collected at surgery performed as part of the treatment either for the removal of nasal polyps or for the resection of hypertrophied turbinates. All medications were prohibited for at least one month prior to surgery. The study was approved by the Nippon Medical School Medical Ethics Committee and informed consent was taken from all patients.

Collection and processing of specimens

Nasal polyp tissues were obtained at surgery done as a part of the treatment for the removal of nasal polyps (polypectomy/functional endoscopic sinus surgery). Nasal mucosal specimens were obtained at surgery (conchotomy) done for the treatment of hypertrophied turbinates. Both nasal polyp specimens and nasal mucosal tissue specimens (3×4 mm) were rinsed in Phosphate buffered saline (PBS) pH 7.6, and then processed for immunohistochemistry, PCR or ELISA as described below.

Real-time PCR analysis for mRNA expression of TSLP, TARC and MDC

By real time PCR we analyzed the mRNA expression of TSLP, TARC and MDC in nasal polyps from atopics, non-atopics and in the allergic nasal mucosa. Total cellular RNA was extracted from nasal mucosa and nasal polyps and purified using an RNeasy mini kit (Qiagen GmbH, Strasse, Germany). cDNAs were prepared from 1 μg of total RNA by reverse transcription at 37°C for 60 minutes and at 95°C for 5 minutes using the High

capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturers' protocols. Quantitative real-time PCR was carried out using the TaqMan assay and Applied Biosystems 7500 Fast Real Time PCR System (Applied Biosystems). The quantitative real-time PCR assay was based on primers that specifically amplify TSLP, TARC and MDC. The primers and fluorogenic probes for TSLP (Hs00263639_m1), MDC (Hs01574247_m1) and TARC (Hs00171074_m1) and β-actin (Hs99999903_m1) were purchased from Applied Biosystems. The amplification efficiency of the specific primers and β-actin were validated in a preliminary experiment. For the PCR analysis, each sample was run in triplicates in separate tubes to permit quantification of TSLP, TARC and MDC genes normalized to β-actin. The PCR condition consisted of initial denaturation step of 95°C for 20 seconds, followed by 50 cycles of amplification at 95°C for 3 seconds and at 60°C for 30 seconds.

Data analysis

Data analysis was performed using 7500 software version 2.0.4 (Applied Biosystems). For quantitative analysis, we used the ΔΔ Ct method according to the manufacturer's instructions (Applied Biosystems: Relative quantitation of gene expression ABI Prism 7700 sequence detection system. User Bulletin 2;1997).[23] The threshold cycles were used to calculate arbitrary mRNA concentrations by the difference of Ct values between samples and calibrator (qPCR Human Reference Total RNA, Clontech Laboratories). The level of TSLP, MDC and TARC mRNA was normalized to the level of β-actin mRNA.

Immunohistochemistry for the detection of TSLP

Nasal polyp and nasal mucosa tissue specimens were first fixed in periodate lysine paraformaldehyde (PLP) and then incubated for 4 hours each in varying gradients of sucrose from 10 to 20% in PBS. Subsequently, the specimens were embedded in OCT compound, frozen in liquid nitrogen and stored at -80°C till further use. To investigate the expression of TSLP and to detect the localization of TSLP in nasal polyps and the nasal mucosa, immunohistochemistry was performed. For immunohistochemistry, frozen sections of PLP fixed specimens at 5 μm thickness were cut using a cryostat (Sakura Finetek, Tokyo, Japan), air-dried for 30 minutes and then fixed in acetone for 10 minutes. Immunohistochemistry was performed using the peroxidase-based Avidin-Biotin Complex (ABC) method (Vectastain ABC kits, Burlingame, CA, USA). Briefly, the acetone fixed sections were rehydrated by incubation in Tris-buffered saline (TBS) pH 7.4. Thereafter, the specimens were incubated in 10% H_2O_2 for 30 minutes to block endogeneous peroxidase. After 2 rinses in TBS, the specimens were incubated in Protein Block Serum (Dako Japan, Tokyo, Japan). After 2 rinses in TBS, the sections were then incubated for 48 hours at 4°C with the primary antibody, the mouse anti-human TSLP (R&D system, Minneapolis, MN, USA) at (10 μg/mL) optimal concentration. Sections were then rinsed twice in TBS and Biotin blocking was done. Subsequently, the sections were incubated in the horse anti-mouse immunoglobulin (Dako Japan) for 30 minutes at room temperature. After 2 rinses in TBS, the sections were incubated in the ABC regent (Dako Japan) for 30 minutes at room temperature followed by incubation in the AEC substrate. The sections were then rinsed twice in distilled water (DW), incubated for 30 seconds in Mayer's hematoxylin and rinsed again twice in DW. Finally, the specimens were mounted in Dako gel. For negative control, the primary antibodies were substituted with an irrelevant isotype matched mouse IgG (IgG2a) (Dako Japan).

Cell counting

The number of positively stained TSLP+ cells was counted under an Olympus microscope in 6 randomly selected visual fields using an objective micrometer in an area of 0.202 mm^2 and at a magnification of 400 HPF. The average of 6 fields was taken as the cell count for that section.

Analysis of TSLP immunoreactivity in epithelial cells

TSLP expression in nasal epithelial cells was scored using an Image J analysis system (National Institute of Mental Health, Bethesda, MD, USA).The density of staining was converted into a numerical value by using image J, a public domain Java image processing program.[24] The expression in the epithelial cells was measured relative to the expression in the lamina propria.

Analysis for the detection of eosinophils and cell counting

Wright-Giemsa staining was used to identify the eosinophils in the nasal polyp and nasal mucosa tissue sections. The number of eosinophils was counted in 6 randomly selected visual fields using an objective micrometer in an area of 0.202 mm^2 and at a magnification of 400 HPF. The average of the 6 fields was taken as the final count for the section.

ELISA analysis for the detection of IgE in nasal polyps

Nasal polyp tissues were homogenized using ultrasonification and high speed centrifugation and the supernatants were collected and stored at -20°C. The levels of IgE in the nasal polyp extracts were measured using the IgE specific ELISA kits (BET, Montogomery, TX, USA). The minimum detectable level of IgE was 15.6 ng/mL.

Statistical analysis

The data presented are expressed as mean±SEM. Statistical significance for intergroup comparisons was determined by the Mann-Whitney *U* test. Differences were considered significant only when the *P* values was less than 0.05. Correlation between the number of TSLP+ cells and eosinophils in the nasal polyps and nasal mucosa as well as between the number of TSLP+ cells and IgE levels in nasal polyps was determined using the Spear-

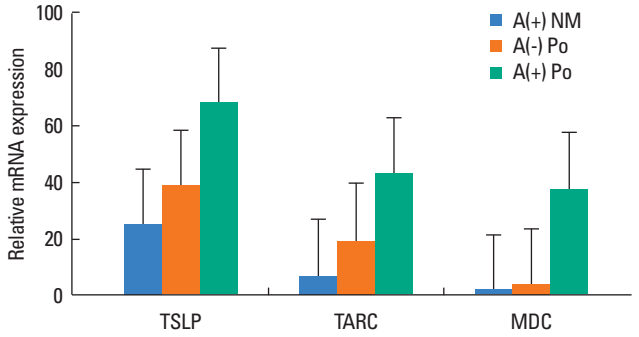

Fig. 1. Relative messenger RNA expression of thymic stromal lymphopoietin (TSLP), thymus and activation-regulated chemokine (TARC) and macrophage derived chemokine (MDC) in nasal polyps (atopic and non-atopic) and allergic nasal mucosa. The mRNA expression was analyzed by real time PCR as described in the text. The quantitative real-time PCR assay was based on primers that specifically amplify TSLP, TARC and MDC. The mRNA expression of TSLP as well as TARC and MDC was markedly higher in nasal polyps from atopics as compared to the allergic nasal mucosa and relatively higher than in nasal polyps from non-atopics.
A+ Po, nasal polyps from atopics (n=4); A- Po, nasal polyps from non-atopics (n=3); A+ NM, nasal mucosa from allergic rhinitis patients (n=3).

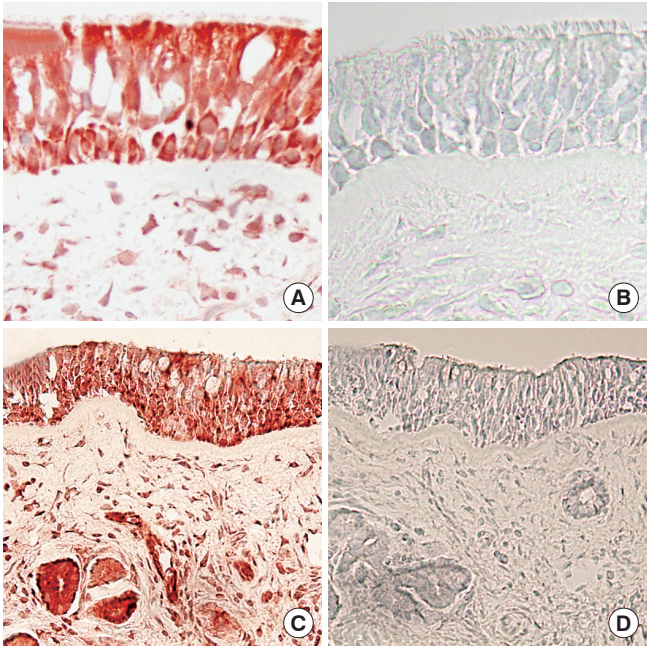

Fig. 2. Immunoreactivity and localization of thymic stromal lymphopoietin (TSLP) in the nasal mucosa. The immunoreactivity for TSLP was analyzed by immunohistochemistry using the peroxidase-based Avidin-Biotin Complex (ABC) method as described in the text. TSLP immunoreactivity is as shown as in (A) in the nasal mucosa of patients with non-allergic rhinitis (NAR). TSLP was expressed strongly especially in epithelial cells. (B) Negative control in NAR shows no immunoreactivity for TSLP. (C) In allergic rhinitis nasal mucosa. TSLP was strongly expressed in epithelial cells, and inflammatory cells. (D) Negative control shows no immunoreactivity for TSLP (magnification ×400 HPF).

man's coefficient. All statistical analysis was performed using the SPSS 11.0 version (SPSS, Inc., Chicago, IL, USA).

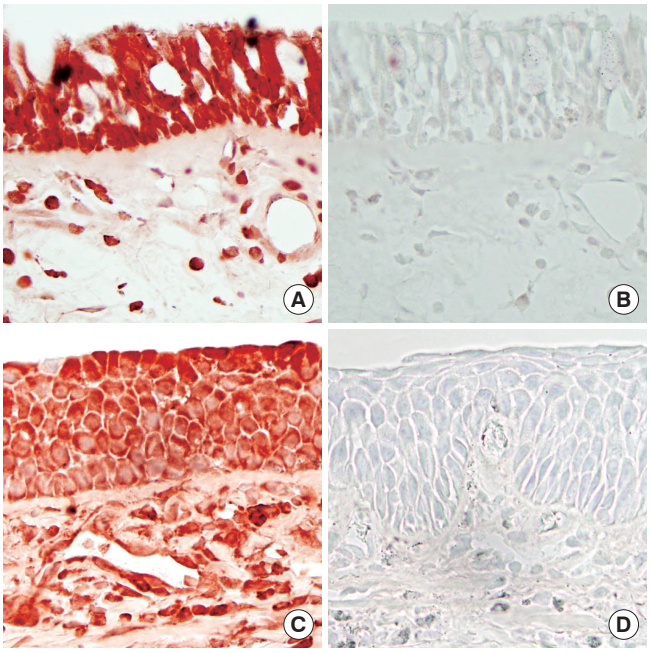

Fig. 3. Immunoreactivity and localization of thymic stromal lymphopoietin (TSLP) in nasal polyps. The immunoreactivity for TSLP was analyzed by immunohistochemistry using the using the peroxidase-based Avidin-Biotin Complex (ABC) method as described in the text. TSLP immunoreactivity is as shown in (A). In nasal polyps from non-atopics, TSLP was expressed strongly especially in epithelial cells. (B) Negative control shows no immunoreactivity for TSLP. (C) In nasal polyps from atopics. TSLP expression was detected in the epithelial cells, endothelial cells, fibroblasts and in inflammatory cells in nasal polyps from both atopics and non-atopics. Stronger immunoreactivity for TSLP was detected in the nasal polyps from atopics. (D) Negative control shows no immunoreactivity for TSLP (magnification ×400 HPF).

RESULTS

Messenger RNA expression of TSLP, TARC and MDC in nasal polyps and nasal mucosa

We analyzed the mRNA expression of TSLP, TARC and MDC in the nasal polyps from atopics, non-atopics and allergic nasal mucosa by real time PCR. Messenger RNA expression of TSLP, TARC and MDC were detected in nasal polyps from atopics, non-atopic nasal polyps and in the allergic nasal mucosa. The mRNA expression of TSLP as well as TARC and MDC was markedly higher in nasal polyps from atopics as compared to the allergic nasal mucosa (Fig. 1) and relatively higher than in nasal polyps from non-atopics.

Immunohistochemical localization of TSLP in the nasal mucosa and nasal polyps

We analyzed the expression of TSLP in the nasal mucosa of patients with AR and NAR. TSLP expression was detected in the epithelial cells, endothelial cells and in inflammatory cells in the nasal mucosa of patients with NAR (Fig. 2A) and AR (Fig. 2C). Negative control did not show any immunoreactivity for

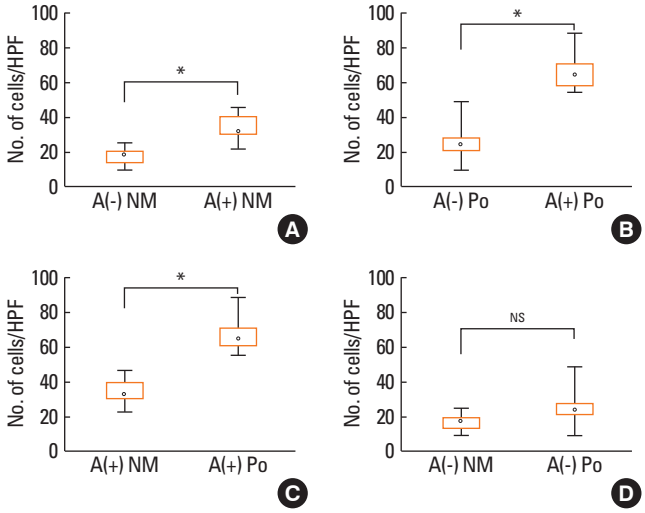

Fig. 4. The number of thymic stromal lymphopoietin (TSLP) positive cells in the nasal mucosa and nasal polyps. Immunohistochemistry for TSLP was performed as described in the text. Positively stained cells were counted as described in the text in an area on 0.202 $mm^2 \times 400$ HPF. (A) The number of TSLP positive cells in the nasal mucosa of patients with allergic rhinitis (AR) was significantly greater than that in the nasal mucosa of patients with non-allergic rhinitis (NAR) (*$P<0.01$). (B) The number of TSLP positive cells in nasal polyps from atopics was significantly greater than that of non-atopics (*$P<0.01$). (C) The number of TSLP positive cells was greater in the nasal polyps of atopics as compared to that of AR nasal mucosa (*$P<0.01$). (D) No significant difference was found in the number of TSLP+ cells between non-atopic nasal polyps and nasal mucosa of NAR patients.
A+ Po, nasal polyps from atopics; A- Po, nasal polyps from non-atopics; A+ NM, nasal mucosa from AR patients; A- NM, nasal mucosa from NAR patients.

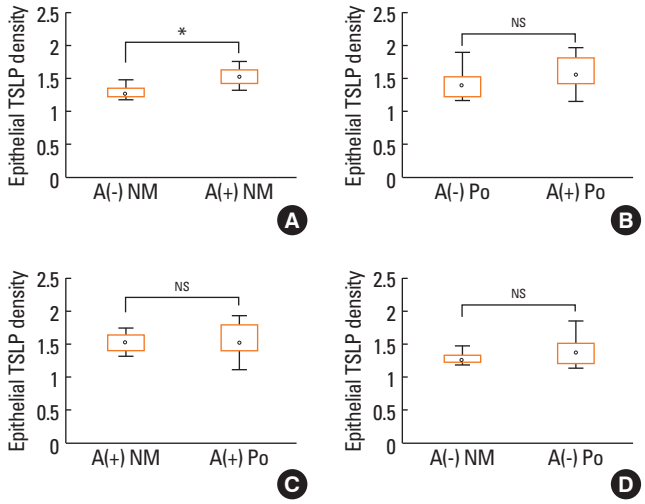

Fig. 5. Epithelial cell expression of thymic stromal lymphopoietin (TSLP) in the nasal mucosa and nasal polyps. TSLP expression in nasal epithelial cells was scored using an Image J analysis system as described in the text. (A) The expression of TSLP in nasal epithelial cells of patients with allergic rhinitis (AR) was significantly greater than that in the nasal mucosa of patients with non-allergic rhinitis (NAR) (*$P<0.05$). (B) TSLP expression in the nasal polyp epithelial cells of atopic patients was slightly higher than that of non-atopics but no significant difference was detected. (C) No difference was detected in the expression of TSLP in epithelial cells of nasal polyps from atopics and nasal mucosa of AR patients. (D) No difference was detected between nasal polyp epithelial cells of non-atopics and nasal epithelial cells of NAR patients.
A+ Po, nasal polyps from atopics; A- Po, nasal polyps from non-atopics; A+ NM, nasal mucosa from AR patients; A- NM, nasal mucosa from NAR patients.

TSLP (Fig. 2B and D). We also analyzed the expression of TSLP in the nasal polyps of atopic patients as well as nasal polyps from non-atopic patients. TSLP expression was detected in the epithelial cells, endothelial cells, fibroblasts and in inflammatory cells in nasal polyps from both non-atopics (Fig. 3A) and atopics (Fig. 3C). Negative control did not show any immunoreactivity for TSLP (Fig. 3B and D).

Numbers of TSLP+ cells in the nasal mucosa and nasal polyps

The number of TSLP positive cells in the nasal mucosa of patients with AR was significantly greater than that in the nasal mucosa of patients with NAR (AR, 33.9±7.4/HPF; NAR, 17.5±5.2/HPF, $P<0.05$) (Fig. 4A). The number of TSLP positive cells in nasal polyp from atopics was significantly greater than that of non-atopics (A+Po, 66.2±10.2/HPF; A-Po, 26.7±10.8/HPF; $P<0.05$) (Fig. 4B). Moreover, the number of TSLP+ cells in nasal polyps from atopics was also greater than that in the nasal mucosa of AR patients (Fig. 4C). No significant difference was found in the number of TSLP+ cells between non-atopic nasal polyps and nasal mucosa of NAR (Fig. 4D).

Expression of TSLP in epithelial cells of nasal polyps and nasal mucosa

The expression of TSLP in nasal epithelial cells of patients with AR was significantly greater than that in the nasal mucosa of patients with NAR (AR, 1.53±0.14; NAR, 1.29±0.10; $P<0.05$) (Fig. 5A). Although the TSLP expression in the nasal polyp epithelial cells of atopic patients was slightly higher than of non-atopics, no significant difference was detected (atopic polyp, 1.58±0.27; non-atopic polyp, 1.41±0.23) (Fig. 5B). No difference was detected in the expression of TSLP in epithelial cells of nasal polyps from atopics and nasal mucosa of AR patients (Fig. 5C) or between nasal polyp epithelial cells of non-atopics and nasal epithelial cells of NAR patients (Fig. 5D).

Eosinophils in nasal polyps

The number of eosinophils in nasal polyps was significantly greater in the nasal polyps as compared to the nasal mucosa of AR patients (nasal polyp, 22.5±27.0/HPF; nasal mucosa of AR patients, 4.33±4.82/HPF; $P<0.001$) Furthermore, the number of eosinophils in nasal polyps of atopics was significantly greater than that in nasal polyps from non-atopics (atopic polyp, 40.6±30.6/HPF; non-atopic polyp, 6.7±6.0/HPF; $P<0.001$).

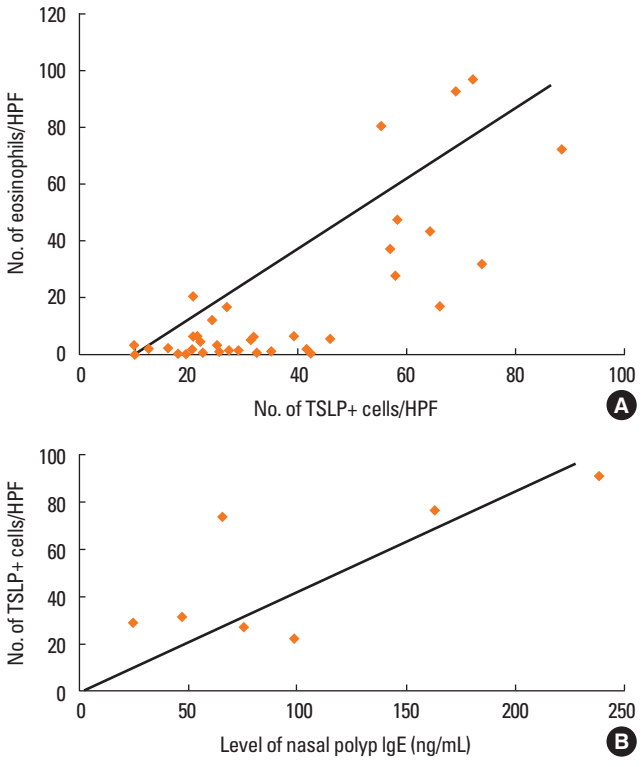

Fig. 6. Correlation between thymic stromal lymphopoietin (TSLP) and eosinophils in nasal polyps. There was a statistically significant correlation between the number of TSLP+ cells (A) and the number of eosinophils in nasal polyps (B) (r =0.78, $P<0.05$).

Level of IgE in nasal polyps by ELISA

The level of IgE in nasal polyps was measured by IgE specific ELISA kits as described in the methods section. The mean level of IgE in nasal polyps was 97.6±75.0 ng/mL.

Correlation between the number of TSLP positive cells and eosinophils in nasal polyps

To understand the relation between TSLP and eosinophilic inflammation in nasal polyps, we analyzed the correlation between TSLP+ cells and number of eosinophils in nasal polyps. There was a statistically significant correlation between the numbers of TSLP+ cells and the number of eosinophils in nasal polyps (r=0.78; $P<0.001$) (Fig. 6).

Correlation between the number of TSLP positive cells and IgE in nasal polyp

There was a statistically significant correlation between the number of TSLP+ cells in nasal polyps and the level of IgE in nasal polyps (r=0.75; $P<0.05$).

DISCUSSION

TSLP plays an important role in the DC-mediated activation of Th2 inflammatory responses.[12,18] In this study, we detected the in vivo expression of TSLP mRNA and protein in nasal polyps of patients with nasal polyposis. We also detected that the expression of TSLP in nasal polyps was significantly greater than that in the allergic nasal mucosa irrespective of whether the nasal polyp patients were atopic or non-atopic. Furthermore, the expression of TSLP was greater in nasal polyps from atopics as compared to those from non-atopics. To the best of our knowledge this is the first report of TSLP expression in nasal polyps.

As reported in atopic dermatitis and asthma, TSLP was mainly localized to the epithelial cells.[17,25] Epithelial cell-derived TSLP can play a major role not only in AR but also in nasal polyps as a master by inducing a dendritic cell-mediated Th2-type allergic inflammation. However, Corrigan et al reported TSLP expression in inflammatory cells in atopic dermatitis.[26] In the present study, immunoreactivity for TSLP was also detected in endothelial cells, fibroblasts and inflammatory cells in nasal polyps.

Although we did not perform double staining in this study, morphologically some of the TSLP+ inflammatory cells appeared to be mast cells. This supposed expression of TSLP in mast cells, coupled with the reports of TSLP+ mast cells in vernal conjunctivitis and allergic conjunctivitis, and consistent with previous studies that mast cells activated through the IgE receptor can express TSLP mRNA support the proposal[27] that mast cells are essential for TSLP expression in the epithelium. In another study TSLP expression was up-regulated predominantly in the nasal epithelium in the ovalbum in (OVA)-sensitized and -nasally challenged mouse model of AR, which was abolished in mast cell-deficient WBB6F1-W/W(v) in comparison with control WBB6F1-+/+ mice and reduced in Fc receptor gamma chain (FcgammaR)-deficient mice, suggesting that mast cell regulation of epithelial TSLP expression, possibly via FcepsilonRI, plays an important role in the development of allergic inflammation.[28] In this context, previously we and others have shown that mast cells are an important source of IL-4, IL-13 and TNF-alpha and that IL-4, IL-13 in concert with TNF-alpha were capable of upregulating TSLP production from nasal polyp fibroblasts.[6] We also showed that nasal polyp fibroblasts in vivo expressed TSLP.[6] Taken together these results show that mast cells in nasal polyps may play an important role in regulating TSLP expression in nasal polyp epithelial cells and fibroblasts. Furthermore, the increased mRNA expression of TSLP in nasal polyps was associated with increased mRNA expression of Th2 type chemokines namely TARC and MDC.

Recently, it was reported that TSLP produced by epithelial cells and keratinocytes can modulate mature CD4+ T-cell proliferation and survival through its interaction with dendritic cells.[29] TSLP stimulates Th2 cytokine production, such as IL-13. Our results demonstrate high expression of TSLP in nasal epithelial cells of AR patients and a stronger expression in nasal polyp epithelial cells. Therefore, TSLP from nasal polyp epithelial cells can activate mast cells to produce Th2 type cytokines like IL-4

and IL-13 that can contribute to eosinophilic inflammation by upregulation of eosinophil chemoattractants and adhesion molecules.[23] We previously showed that epithelial cells express HLA-DR and CD86 and Fcepsilon receptor alpha chain and that IL-4 can upregulate Fcepsilon alpha chain expression in nasal epithelial cells indicating an augmented antigen presenting activity.[30] Furthermore, the increased mRNA expression of TSLP in nasal polyps was associated with increased mRNA expression of other Th2 type chemokines namely TARC and MDC.[5,24]

Emerging data have shown that the expression of TSLP was elevated in asthmatic airway. Besides, studies have revealed in animal model, the up-regulation of TSLP in nasal mucosa. Our study demonstrates that the excessive expression of TSLP in epithelial cells of patients with AR and nasal polyposis. TSLP produced by the epithelial interface, and may represent a mechanism whereby environmental stimuli initiate Th2 responses to allergen, and through chemokine production retaining or attracting in asthmatic airways.[20]

Eosinophilic nasal polyps is characterized by a polarized Th2 reaction and eosinophilic inflammation.[1,2,31] To further investigate whether TSLP is associated in regulating eosinophil infiltration in nasal polyps, Wright Giemsa staining was performed to evaluate the numbers of eosinophils in the nasal polyps and the correlation between the number of TSLP+ cells and eosinophil numbers was analyzed. Our study showed a strong correlation between the numbers of eosinophils in nasal polyps and numbers of TSLP+ cells. This result support the hypothesis that epithelial cells sourced TSLP may have a potential role in regulating eosinophilic inflammation in the nasal polyps possibly via driving Th2 type inflammation that can upregulate eosinophilic chemoattractants. While the relationship between TSLP and tissue eosinophilia deserves further study, the roles of TSLP in the survival and recruitment of mast cells in airway inflammation is also interesting and need further studies.

In nasal polyps, high IgE concentrations have been reported indicating that IgE is produced locally.[32,33] Van Zele et al.[32] also reported a polyclonal hyper-immunoglobulinemia E, associated with the presence of IgE specific to Staphylococcus aureus enterotoxins, colonization with Staphylococcus aureus and increased eosinophilic inflammation in a relevant subgroup of nasal polyp patients (about 50%). They also reported a strong correlation between IgE in nasal polyps and nasal eosinophils. Our results demonstrated high levels of IgE in nasal polyps and a good correlation between the levels of nasal polyp IgE and the number of TSLP+ cells in nasal polyps as well as between TSLP and eosinophils.

Mast cells release mediators upon stimulation that contribute to the pathogenesis of chronic airway disease, including the recruitment and activation of Th2 lymphocytes. We previously demonstrated that mast cells can release IL-4 and IL-13 express the CD40 ligand and induce IgE synthesis locally in the nasal mucosa and that this was more IL-13 dependant.[34]

Allakhverdi et al.[25] showed partial suppression of IL-13 expression using anti-TSLP antibody in a mast cell-lesional, AD-skin coculture model suggesting the role of TSLP in enhancing IL-13 production.

Sheahan et al.[35] demonstrated local IgE production in non-atopic nasal polyposis also.

In summary, we found the increased *in vivo* expression of TSLP in the epithelial cells and infiltrating cells of patients with nasal polyps irrespective of their atopic status. The presence of Th2 type inflammation in nasal polyps from both atopic and non-atopic patients as well as a more severe form of Th2 type inflammation in nasal polyps as compared to the allergic nasal mucosa could in part be due to the differential and upregulated expression of TSLP in nasal polyps. These findings indicate a potential and crucial role for TSLP in role in the pathogenesis of severe chronic inflammation in nasal polyps via regulating the Th2 and eosinophilic inflammation.

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