

Thymic stromal lymphopoietin is a critical mediator of IL-13-driven allergic inflammation

Masanori Miyata^{1,2}, Yuki Nakamura¹, Naomi Shimokawa¹,
Yuko Ohnuma¹, Ryohei Kato³, Shuji Matsuoka⁴, Ko Okumura⁵,
Hideoki Ogawa⁵, Keisuke Masuyama² and Atsuhito Nakao^{1,5}

¹ Department of Immunology, University of Yamanashi Faculty of Medicine, Yamanashi, Japan

² Department of Otorhinolaryngology, Head and Neck Surgery, University of Yamanashi Faculty of Medicine, Yamanashi, Japan

³ Department of Human Pathology University of Yamanashi Faculty of Medicine, Yamanashi, Japan

⁴ Department of Pathology, Juntendo University School of Medicine, Tokyo, Japan

⁵ Atopy Research Center, Juntendo University School of Medicine, Tokyo, Japan

Both thymic stromal lymphopoietin (TSLP) and IL-13 are essential cytokines for the development of allergic inflammation. However, a causal link between TSLP and IL-13 has not yet been fully elucidated. This study aimed to investigate whether IL-13 induces TSLP expression and whether the induction contributes to the development of allergic inflammation. We found that IL-13 induced TSLP expression in mouse nasal tissue specimens in a Stat6-dependent manner. In addition, intranasal challenge of mice with IL-13 induced TSLP expression in the nasal epithelium. Importantly, intranasal IL-13 challenge induced eosinophilia and goblet cell hyperplasia in the nasal mucosa in mice, which was inhibited by the blockade of TSLP activity with anti-TSLP Ab. These findings suggest that TSLP is an important mediator of IL-13-driven allergic inflammation in the nasal mucosa. Taken together with the recent findings that IL-13 is a critical downstream element for TSLP-driven allergic inflammation, TSLP may function both upstream and downstream of IL-13, thus providing an additional rationale as to why TSLP plays such a central role in the development of allergic inflammation.

Key words: Allergic inflammation · Epithelial cells · IL-13 · Thymic stromal lymphopoietin



Supporting Information available online

Introduction

Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine, which binds to a TSLP receptor (TSLPR) consisting of the IL-7 receptor α -chain and a common γ receptor-like chain [1, 2]. TSLP was originally identified as a factor derived from a thymic stromal cell line that could support the growth of a mouse B-cell line [3].

However, recent evidence has demonstrated TSLP, derived from epithelial cells, to be a critical factor for allergic inflammation [1, 2, 4]. For instance, transgenic mice expressing TSLP in keratinocytes or in lung epithelial cells develop atopic dermatitis- or asthma-like inflammation in the skin or the lung, respectively, while TSLPR-deficient mice fail to develop an inflammatory lung response to inhaled antigen [5–7].

IL-13, a Th2-type cytokine, is a central mediator for allergic inflammation [8, 9]. The blockade of IL-13 markedly inhibits allergen-induced airway hyperresponsiveness, mucus production, and eosinophilia whereas IL-13 delivery to the airway causes

Correspondence: Dr. Atsuhito Nakao
e-mail: anakao@yamanashi.ac.jp

these effects in mice [8–11]. Therefore, IL-13 as well as TSLP is necessary and sufficient for the development of allergic inflammation. However, the relationship between TSLP and IL-13 in allergic inflammation has not yet been fully elucidated.

This study thus investigated the causal link between IL-13 and TSLP in allergic inflammation. For this purpose, we examined the effects of IL-13 on TSLP expression both *in vitro* and *in vivo* using mouse nasal tissue explants and a mouse model of allergic inflammation in the nasal mucosa, respectively. The current results suggest that TSLP is induced by IL-13 and is critical for the development of IL-13-driven allergic inflammation. Because recent studies suggest that TSLP-driven skin and lung allergic inflammation relies on intact IL-4/IL-13 responses [12, 13], the roles of TSLP in the development of allergic inflammation are herein also discussed by attempting to reconcile these contrasting results.

Results and discussion

IL-13 induces TSLP expression in normal nasal tissue in a Stat6-dependent manner

To investigate whether IL-13 can induce TSLP expression, the effects of IL-13 on TSLP expression were first examined using nasal tissue explants isolated from WT mice.

The nasal tissue specimens were stimulated with IL-13 *in vitro* for 24 h and analyzed for TSLP protein levels. IL-13 induced TSLP production in the nasal tissue specimens in a dose-dependent manner (Fig. 1A). An immunohistochemical analysis showed TSLP expression to be predominantly detected in the nasal epithelium of the tissue specimens (Fig. 1B). In addition, the nasal tissue specimens derived from mast cell-deficient W/Wv mice significantly produced TSLP upon IL-13 stimulation (Fig. 1C), thus suggesting that TSLP was not produced by mast cells present in the nasal tissue. The nasal tissue specimens derived from Myd88-deficient mice also produced TSLP upon IL-13 stimulation (Fig. 1D), thus suggesting that any possible LPS contamination in recombinant IL-13 reagent or during experimental procedures was not involved in the IL-13-induced TSLP production. Importantly, IL-13-induced TSLP production was abrogated in the nasal tissue explants obtained from Stat6-deficient mice (Fig. 1E).

To further investigate whether IL-13 induced TSLP production *in vivo*, the WT mice were intranasally challenged with IL-13 and nasal tissue samples were collected for subsequent evaluations. An immunohistochemical analysis showed that the IL-13 challenge induced TSLP expression predominantly in epithelial cells in the nasal mucosa (Fig. 2A). These results suggested that IL-13 induced TSLP expression in the nasal epithelium.

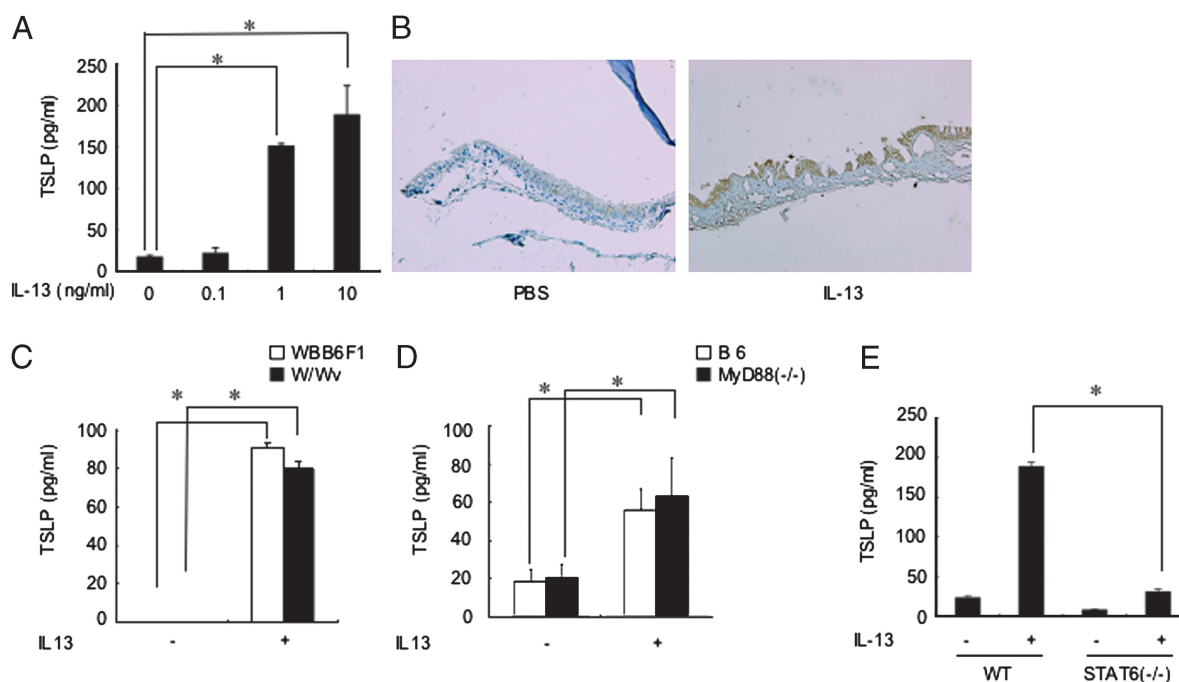


Figure 1. IL-13 induces TSLP expression in mouse nasal tissue. (A) Nasal tissue explants isolated from BALB/c mice were stimulated with 0.1, 1, 10 ng/mL of IL-13 for 24 h. Culture supernatants were collected and TSLP concentrations were determined by ELISA. (B) Representative image of nasal tissue explants stimulated with PBS or 10 ng/mL IL-13 and stained with anti-TSLP Ab as described in the Materials and methods. (C–E) Nasal tissue explants isolated from mast cell-deficient WBB6F1-W/Wv and control WBB6F1^{+/+} mice (C) (*n* = 3), Myd88-deficient and control C57BL/6 mice (D) (*n* = 6), or Stat6-deficient and control BALB/c mice (*n* = 3) (E) were stimulated with 10 ng/mL of IL-13 for 24 h. Culture supernatants were collected and TSLP concentrations were determined by ELISA. Data show mean ± SD. **p* < 0.05, Mann–Whitney *U* test (A and C–E). Representative results from three independent experiments are shown (A–E).

IL-13-driven allergic inflammation depends on TSLP expression

To further investigate whether IL-13-induced epithelial TSLP expression plays an important role in the development of IL-13-driven allergic inflammation, we examined the effects of anti-TSLP neutralizing Ab on the development of IL-13-driven allergic inflammation in mouse nasal mucosa.

Intranasal exposure of IL-13 to the mice described in Fig. 2A induced eosinophilia and goblet cell hyperplasia in the nasal mucosa (Fig. 2B and C). The expression of eosinophil-associated RNase II (mEAR2), a marker of eosinophils [14], and Mucin-5/5ac, a marker of goblet cell hyperplasia [15], mRNA also increased as a result of IL-13 exposure in the nasal tissue (Fig. 2D). Importantly, administration of anti-TSLP neutralizing Ab, but not control Ab, during IL-13 challenge significantly inhibited the development of eosinophilia and goblet-cell hyperplasia in the nasal mucosa associated with a reduced mEAR2 and Mucin-5/5ac mRNA expression (Fig. 2B–D). These results suggested that IL-13-induced epithelial TSLP expression was critical for the development of IL-13-driven allergic inflammation in the nasal mucosa in mice.

IL-13 induces TSLP expression in normal trachea, lung, and skin tissue

To determine whether IL-13-induced TSLP production is specific to nasal tissue, other mouse tissue samples were tested. IL-13 induced TSLP production in the trachea, lung, and skin tissue specimens, but not in small and large intestinal tissue specimens, isolated from WT mice, which was abrogated by Stat6-deficiency (Supporting Information Fig. 1A). Thus, IL-13-induced TSLP production may be tissue-specific. In addition, IL-13 induced TSLP production in the trachea, lung, and skin tissue specimens derived from mast cell-deficient W/W^v mice and Myd88-deficient mice (Supporting Information Fig. 1B and C), which was consistent with the results observed in the nasal tissue specimens (Fig. 1).

Previous studies have suggested that TSLP is induced by proinflammatory cytokines (e.g. TNF- α or IL-1 α), TLR ligands, and vitamin D in primary human bronchial epithelial cells and keratinocytes [16–20]. In such cell types, IL-13 alone did not induce TSLP production in contrast to the current results, although IL-13 was able to induce TSLP production in synergy with proinflammatory cytokines such as TNF- α [18–20]. The difference from the current results may be explained by the use of tissue explants in this study. Bogiatzi *et al.* reported that TSLP expression was detected at the protein level when using whole skin explants, but it was not detected using a primary keratinocyte culture system [19]. Consistently, we found that IL-13, even in the presence of TNF- α , did not induce TSLP expression at the protein level in the human nasal epithelial cell line RPMI 2650 (our unpublished data). Alternatively, because this study used the tissue explants containing other cell types than epithelial cells, the major cellular source of TSLP in response to IL-13 may not be airway or skin epithelial cells and lead to the different results. Although the immunohistochemical studies showed predominant

TSLP staining in the mouse nasal epithelium (Fig. 1B and Fig. 2A), it is still possible that TSLP may be released by other cells (e.g. stromal cells) and bind to the epithelium. This issue will be clarified in our future studies.

In addition, Bogiatzi *et al.* reported that TSLP was not induced by IL-13 alone in their human skin explants [19], which was in contrast to the current results in the mouse skin explants (Supporting Information Fig. 1). The discrepancy could be attributed to the use of tissue samples derived from different species. Some TSLP effects are likely to differ between humans and mice [1].

The basal levels of TSLP production were reproducibly observed in nasal, trachea, lung, and skin explants cultured without IL-13 stimulation (Fig. 1 and Supporting Information Fig. 1), which was consistent with the findings observed in human skin explants [21]. Endogenous stimulators of TSLP production might be released upon the cellular damage associated with the tissue isolation and contribute to the basal TSLP production. This issue is presently under investigation.

IL-13 plays a central role in the development of airway inflammation in asthma and allergic rhinitis [8–11, 22–24]. Recent studies suggested that TSLP functions upstream of IL-13 in the skin and lung allergic inflammation [12, 13]. Given the current findings, TSLP may act both upstream and downstream of IL-13 and contribute to the initiation and maintenance of allergic inflammation; TSLP acting upstream of IL-13 may play an important role in the differentiation of Th2 type cells or in the activation of mast cells to produce IL-13 [1, 2, 4, 21] whereas TSLP acting downstream of IL-13 may play an important role in the regulation of Th2 cell function [25], in the maintenance of memory Th2 cells [26], and in the production of chemokines associated with inflammation [27] (Fig. 3). Thus, TSLP and IL-13 may form a positive feedback loop to drive allergic inflammation as also suggested by previous studies [12, 18].

Concluding remarks

IL-13 alone was able to induce TSLP production in the nasal epithelium and the induction was critical for the development of IL-13-driven allergic inflammation in the nasal mucosa in mice. These results may provide an additional rationale as to why TSLP plays such a central role in the development of allergic inflammation.

Materials and methods

Mice

Female 4–6-wk-old BALB/c, C57BL/6, WBB6F1-+/+, and mast cell-deficient WBB6F1-W/W^v mice were purchased from Japan SLC (Tokyo, Japan), Stat6-deficient mice [28] on BALB/c background were provided by Dr. Hiroshi Nakajima (Chiba University, Chiba, Japan), Myd88-deficient mice [29] on C57BL/6 background were purchased from Oriental Yeast (Tokyo, Japan), and these mice were bred under specific pathogen-free conditions. All animal experiments

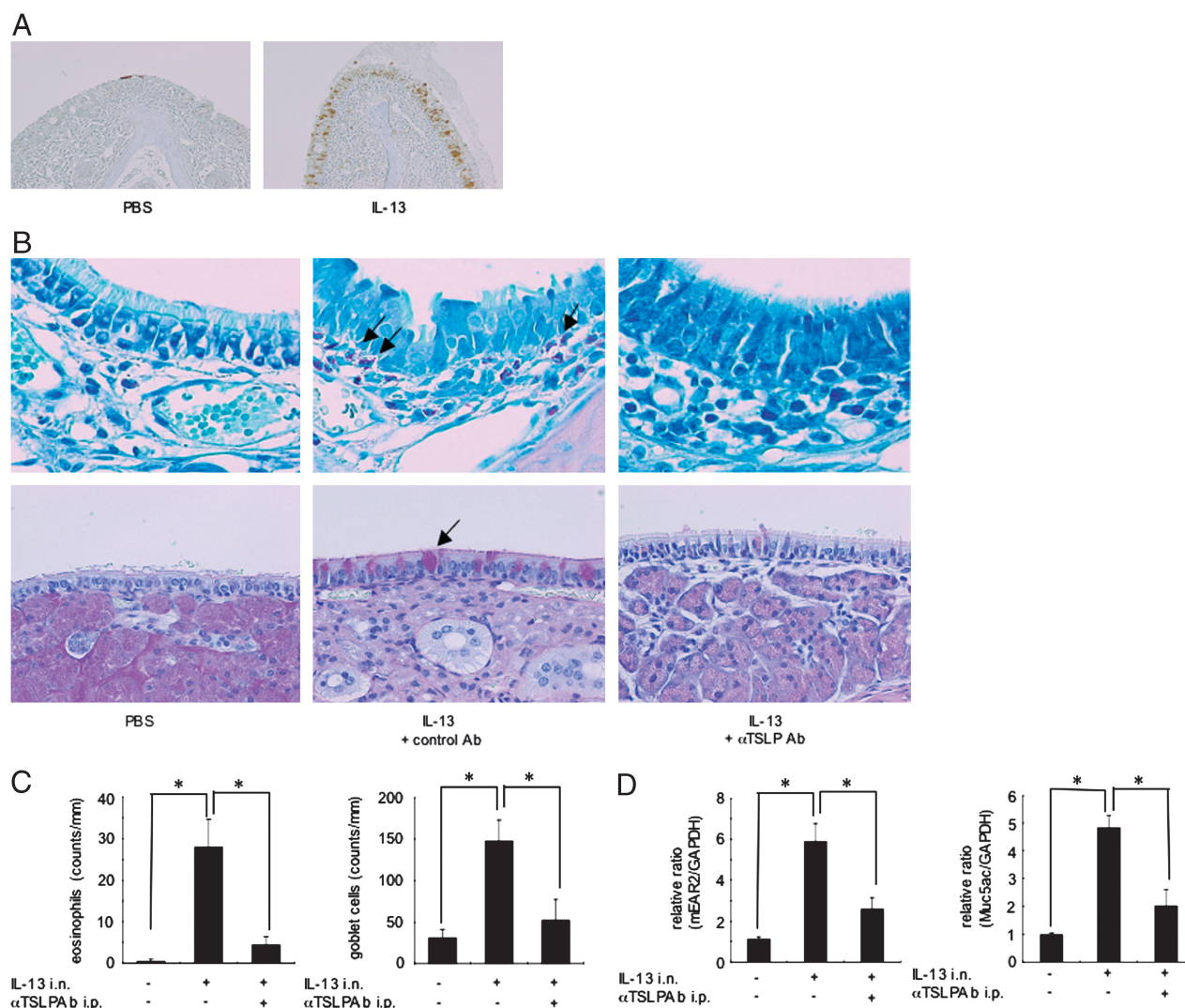


Figure 2. Anti-TSLP neutralizing Ab inhibits the development of IL-13-driven allergic inflammation in the nasal mucosa in mice. Mice were intranasally challenged with 1 μ g IL-13 in 10 μ L PBS or PBS only every 24 h for a total of three times and were sacrificed 24 h after the last IL-13 challenge. To block the endogenous TSLP activity, anti-TSLP neutralizing Ab (α TSLP Ab) or the control Ab (control Ab) was administered intraperitoneally 24 h before the first IL-13 challenge (B–D). (A) Representative images of TSLP expression in the nasal mucosa of mice intranasally challenged with IL-13 or PBS. The positive staining (brown color) is detected predominantly in the nasal epithelium. (B) Representative images of H&E staining (upper panels) or PAS staining (lower panels) of the nasal mucosa specimens obtained from the mice intranasally challenged with PBS or IL-13 treated with anti-TSLP Ab (α TSLP Ab) or control IgG (control Ab). The arrows indicate the H&E- or PAS-positive cells. (C) A quantitative analysis of H&E-positive eosinophils or PAS-positive goblet cells in the nasal mucosa. Data show mean $\pm$ SD ($n = 6$ per each group). * $p < 0.05$, Mann–Whitney U test. (D) Real-time PCR for mEAR2 and Muc5/5ac expression in the nasal tissue obtained from the mice challenged with IL-13 or control PBS treated with anti-TSLP Ab (α TSLP Ab) or control IgG (control Ab). ($n = 6$ per each group). * $p < 0.05$. (Mann–Whitney U test). Representative results from two independent experiments are shown (A–D).

were approved by the Institutional Review Board of the University of Yamanashi.

Culture of mouse tissue specimens

Following the pulmonary and systemic circulation of the mice rinsed with PBS, nasal tissue specimens were isolated from the mouse septum, cut squarely (5 mm $\times$ 5 mm), and were maintained in DMEM (Invitrogen-Gibco, Carlsbad, CA, USA) containing 10% FBS and antibiotics. Trachea, lung, skin, and small and large intestinal

tissue specimens were also isolated from the mice and cultured in DMEM with 10% FBS and antibiotics. For the ELISA, mouse recombinant IL-13 (final 10 ng/mL; R&D Systems, Minneapolis, MN) was added into the *ex vivo* culture for the indicated times.

ELISA

The amounts of TSLP in the culture supernatants of the nasal tissue, trachea, or lung explants were determined using the mouse TSLP ELISA kits (R&D systems) according to the manufacturer's instructions.

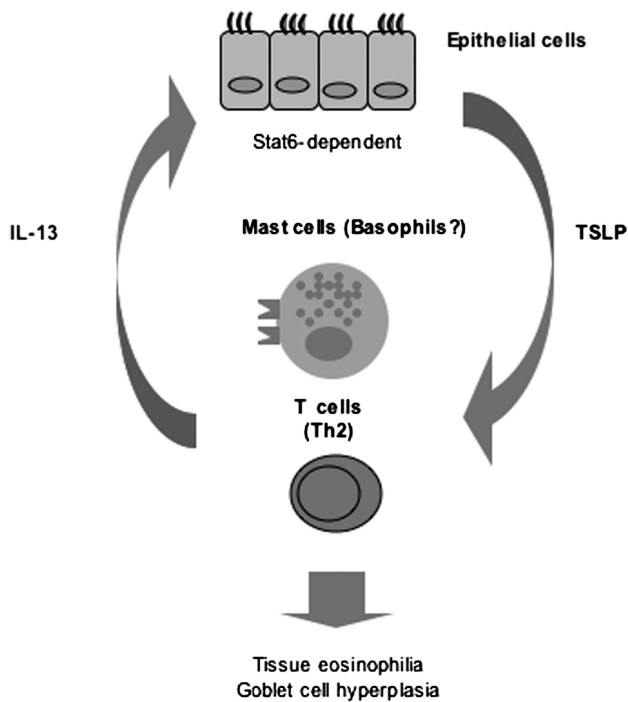


Figure 3. TSLP may act both upstream and downstream of IL-13 in allergic inflammation. Previous findings suggest that IL-13 is an important mediator for TSLP-driven allergic inflammation, which can be attributed to the effects of TSLP on Th2-cell differentiation or mast-cell activation. The current results suggest that TSLP is a downstream target of IL-13/Stat6 pathway and the IL-13-induced TSLP expression is required for the development of allergic inflammation. Thus, TSLP and IL-13 may form a positive feedback loop to drive and maintain allergic inflammation.

Induction of IL-13-driven allergic inflammation in the nasal mucosa

IL-13-driven allergic inflammation in the nasal mucosa was established according to a previously established protocol used in mouse models of asthma [8]. Briefly, the mice were challenged intranasally with 1 μ g of IL-13 in 10 μ L of PBS every 24 h for a total of three times. The mice were challenged intranasally with PBS in a similar manner for the negative control. For some experiments, rat anti-mouse TSLP neutralizing Ab (R&D Systems) or control rat IgG2a (15 mg/kg *per* mouse; Santa Cruz Biotechnology, Santa Cruz, CA, USA) were administered intraperitoneally 24 h before the first IL-13 challenge. The dosage of the Ab (15 mg/kg) was chosen based on previous experiments [30, 31].

Histological examination

Twenty-four hours after the final nasal IL-13 challenge, the mice were killed with carbon dioxide. The heads were removed, fixed, and decalcified. Coronal nasal sections were then visualized by staining with Hansel solution (to demonstrate eosinophils), or periodic acid Schiff (PAS)/hematoxylin (to demonstrate goblet cells). For immunohistochemistry, the coronal nasal sections

were deparaffinized and stained with either anti-TSLP Ab or control IgG Ab (both from Santa Cruz Biotechnology) through the use of peroxidase-based VECTASTAIN ABC kits with DAB substrate (Vector Laboratories, Burlingame, CA, USA).

Quantification of the histological examinations

The number of Hansel-positive eosinophils in the nasal mucosa or the number of PAS-positive goblet cells in the total nasal epithelial lining of the coronal sections was counted microscopically in a blinded manner and it was expressed as the number *per* total length of the total nasal epithelial lining of the coronal sections (the number/mm) as previously described [31]. Two or four specimens of the Hansel- or PAS-stained coronal sections from one mouse were selected. The mean score was calculated, and then the mean scores were calculated in six animals.

Quantitative real-time PCR

Quantitative real-time PCR with specific primers and probes for mouse eosinophil-associated RNase II (mEAR2) [14], Muc-5/5ac [15], and GAPDH (Applied Biosystems, Foster City, CA, USA) was performed using the AB7300 real-time PCR system (Applied Biosystems) as previously described [30].

Data analysis

Values represent the mean $\pm$ SD. Statistical analysis was performed using the non-parametric Mann–Whitney *U* test to compare data in different two groups. A value of $p < 0.05$ was considered to be significant.

Acknowledgements: The authors thank Drs. Akira Suto and Hiroshi Nakajima for providing us with Stat6-deficient mice and Ms. Mutsuko Hara for valuable general assistance. This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

References

- 1 Ziegler, S. F. and Liu, Y. J., Thymic stromal lymphopoietin in normal and pathogenic T cell development and function. *Nat. Immunol.* 2006. 7: 709–714.
- 2 Leonard, W. J., TSLP: finally in the limelight. *Nat. Immunol.* 2002. 3: 605–607.

- 3 Sims, J. E., Williams, D. E., Morrissey, P. J., Garka, K., Foxworthe, D., Price, V., Friend, S. L. et al., Molecular cloning and biological characterization of a novel murine lymphoid growth factor. *J. Exp. Med.* 2000. **192**: 671–680.
- 4 Liu, Y. J., Thymic stromal lymphopoietin: master switch for allergic inflammation. *J. Exp. Med.* 2006. **203**: 269–273.
- 5 Yoo, J., Omori, M., Gyarmati, D., Zhou, B., Aye, T., Brewer, A., Comeau, M. R. et al., Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. *J. Exp. Med.* 2005. **202**: 541–549.
- 6 Zhou, B., Comeau, M. R., De Smedt, T., Liggitt, H. D., Dahl, M. E., Lewis, D. B., Gyarmati, D. et al., Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat. Immunol.* 2005. **6**: 1047–1053.
- 7 Al-Shami, A., Spolski, R., Kelly, J., Keane-Myers, A. and Leonard, W. J., A role for TSLP in the development of inflammation in an asthma model. *J. Exp. Med.* 2005. **202**: 829–839.
- 8 Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T. Y., Karp, C. L. and Donaldson, D. D., Interleukin-13: central mediator of allergic asthma. *Science* 1998. **282**: 2258–2261.
- 9 Grünig, G., Warnock, M., Wakil, A. E., Venkayya, R., Brombacher, F., Rennick, D. M., Sheppard, D. et al., Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998. **282**: 2261–2263.
- 10 Zhu, Z., Homer, R. J., Wang, Z., Chen, Q., Geba, G. P., Wang, J., Zhang, Y. and Elias, J. A., Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest.* 1999. **103**: 779–788.
- 11 Kuperman, D. A., Huang, X., Koth, L. L., Chang, G. H., Dolganov, G. M., Zhu, Z., Elias, J. A. et al., Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat. Med.* 2002. **8**: 885–889.
- 12 Zhou, B., Headley, M. B., Aye, T., Tocker, J., Comeau, M. R. and Ziegler, S. F., Reversal of thymic stromal lymphopoietin-induced airway inflammation through inhibition of Th2 responses. *J. Immunol.* 2008. **181**: 6557–6562.
- 13 Jessup, H. K., Brewer, A. W., Omori, M., Rickel, E. A., Budelsky, A. L. and Yoon, B. R., Intradermal administration of thymic stromal lymphopoietin induces a T cell- and eosinophil-dependent systemic Th2 inflammatory response. *J. Immunol.* 2008. **181**: 4311–4319.
- 14 McDevitt, A. L., Deming, M. S., Rosenberg, H. R. and Dyer, K. D., Gene structure and enzymatic activity of mouse eosinophil-associated ribonuclease 2. *Gene* 2001. **267**: 23–30.
- 15 Zuhdi Alimam, M., Piazza, F. M., Selby, D. M., Letwin, N., Huang, L. and Rose, M. C., Muc-5/5ac mucin messenger RNA and protein expression is a marker of goblet cell metaplasia in murine airways. *Am. J. Respir. Cell. Mol. Biol.* 2000. **22**: 253–260.
- 16 Holgate, S. T., The epithelium takes center stage in asthma and atopic dermatitis. *Trends Immunol.* 2007. **28**: 248–251.
- 17 Li, M., Hener, P., Zhang, Z., Kato, S., Metzger, D. and Chambon, P., Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. *Proc. Nat. Acad. Sci. USA* 2006. **103**: 11736–11741.
- 18 Kato, A., Favoreto, S., Jr., Avila, P. C. and Schleimer, R. P., TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J. Immunol.* 2007. **179**: 1080–1087.
- 19 Bogiatzi, S. I., Fernandez, I., Bichet, J. C., Marloie-Provost, M. A., Volpe, E., Sastre, X. and Soumelis, V., Cutting Edge: Proinflammatory and Th2 cytokines synergize to induce thymic stromal lymphopoietin production by human skin keratinocytes. *J. Immunol.* 2007. **178**: 3373–3377.
- 20 Soumelis, V., Reche, P. A., Kanzler, H., Yuan, W., Edward, G., Homey, B., Gilliet, M. et al., Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat. Immunol.* 2002. **3**: 673–680.
- 21 Allakhverdi, Z., Comeau, M. R., Jessup, H. K., Yoon, B. R., Brewer, A., Chartier, S., Paquette, N. et al., Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J. Exp. Med.* 2007. **204**: 253–258.
- 22 Miyahara, S., Miyahara, N., Matsubara, S., Takeda, K., Koya, T. and Gelfand, E. W., IL-13 is essential to the late-phase response in allergic rhinitis. *J. Allergy Clin. Immunol.* 2006. **118**: 1110–1116.
- 23 Pawankar, R. U., Okuda, M., Hasegawa, J., Suzuki, K., Yssel, H., Okubo, K., Okumura, K. and Ra, C., Interleukin-13 expression in the nasal mucosa of perennial allergic rhinitis. *Am. J. Respir. Crit. Care Med.* 1995. **152**: 2059–2067.
- 24 Ghaffar, O., Laberge, S., Jacobson, M. R., Lowhagen, O., Rak, S., Durham, S. R. and Hamid, Q., IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. *Am. J. Respir. Cell Mol. Biol.* 1997. **17**: 17–24.
- 25 He, R., Oyoshi, M. K., Garibyan, L., Kumar, L., Ziegler, S. F. and Geha, R. S., TSLP acts on infiltrating effector T cells to drive allergic skin inflammation. *Proc. Natl. Acad. Sci. USA* 2008. **105**: 11875–11880.
- 26 Wang, Y. H., Ito, T., Wang, Y. H., Homey, B., Watanabe, N., Martin, R., Barnes, C. J. et al., Maintenance and polarization of human TH2 central memory T cells by thymic stromal lymphopoietin-activated dendritic cells. *Immunity* 2006. **24**: 827–838.
- 27 Ohba, T., Haro, H., Ando, T., Koyama, K., Hatsushika, K., Suenaga, F., Ohnuma, Y. et al., potential role of thymic stromal lymphopoietin in the recruitment of macrophages to mouse intervertebral disc cells via monocyte chemotactic protein 1 induction: implications for herniated discs. *Arthritis Rheum.* 2008. **58**: 3510–3519.
- 28 Takeda, K., Tanaka, T., Shi, W., Matsumoto, M., Minami, M. and Kashiwamura, S., Essential role of Stat6 in IL-4 signalling. *Nature* 1996. **380**: 627–630.
- 29 Adachi, O., Kawai, T., Takeda, K., Matsumoto, T., Tsutsui, H., Sakagami, M., Nakanishi, K. and Akira, S., Targeted disruption of the Myd88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* 1998. **9**: 143–150.
- 30 Nakamura, Y., Miyata, M., Ohba, T., Ando, T., Hatsushika, K., Suenaga, F., Shimokawa, N. et al., Cigarette smoke extract induces thymic stromal lymphopoietin expression, leading to T(H)2-type immune responses and airway inflammation. *J. Allergy Clin. Immunol.* 2008. **122**: 1208–1214.
- 31 Miyata, M., Hatsushika, K., Ando, T., Shimokawa, N., Ohnuma, Y., Katoh, R., Suto, H. et al., Mast cell regulation of epithelial TSLP expression plays an important role in the development of allergic rhinitis. *Eur. J. Immunol.* 2008. **38**: 1487–1492.

Abbreviations: PAS: periodic acid Schiff · TSLP: thymic stromal lymphopoietin · TSLPR: thymic stromal lymphopoietin receptor

Full correspondence: Dr. Atsuhito Nakao, Department of Immunology, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan
Fax: +81-55-273-9542
e-mail: anakao@yamanashi.ac.jp

Supporting Information for this article is available at
www.wiley-vch.de/contents/jc_2040/2009/39302_s.pdf

Received: 1/2/2009
Revised: 7/7/2009
Accepted: 22/7/2009

European Journal of Immunology

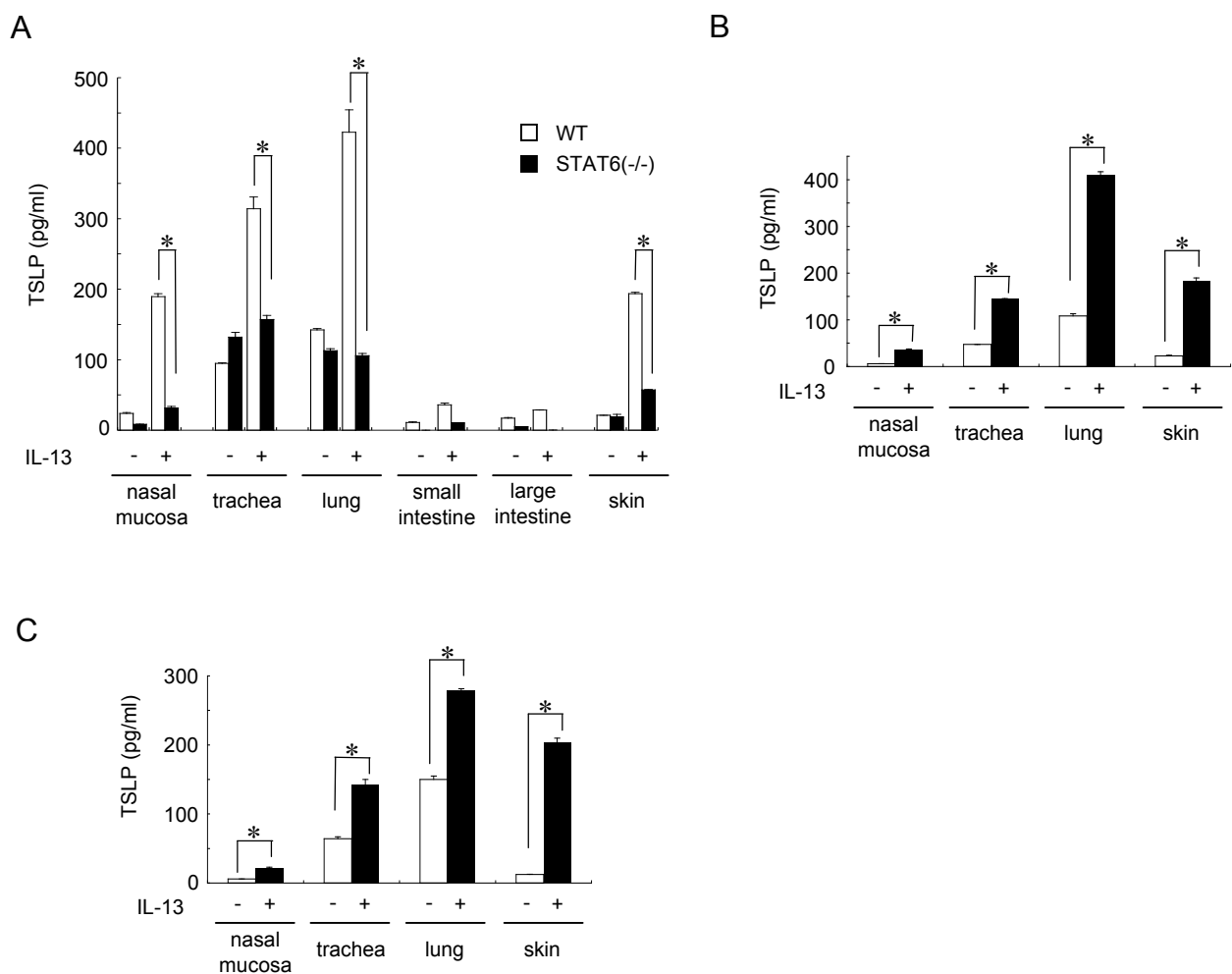
Supporting Information

for

DOI 10.1002/eji.200939302

**Thymic stromal lymphopoietin is a critical mediator
of IL-13-driven allergic inflammation**

Masanori Miyata, Yuki Nakamura, Naomi Shimokawa, Yuko Ohnuma, Ryohei Katoh,
Shuji Matsuoka, Ko Okumura, Hideoki Ogawa, Keisuke Masuyama and
Atsuhito Nakao



Supporting information Figure 1

IL-13 induces TSLP expression in the normal trachea, lung, and skin tissue.

A. Nasal, trachea, lung, skin, and small and large intestinal tissue explants isolated from BALB/c mice and Stat6-deficient mice were stimulated with 10 ng/ml of IL-13 for 24 hr.

B, C. Nasal, trachea, lung, and skin tissue explants isolated from mast cell-deficient W/W^v mice (B) or Myd88-deficient mice (C) were stimulated with 10 ng/ml of IL-13 for 24 hr.

Culture supernatants were then collected and the TSLP concentrations were determined by ELISA. Values represent the mean $\pm$ SD (n = 3 per group). *p < 0.05 (Mann-Whitney U test) (A-C). Representative results from 3 independent experiments are shown (A-C).