

Thymic stromal lymphopoietin: master switch for allergic inflammation

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Thymic stromal lymphopoietin (TSLP) is an interleukin (IL) 7–like cytokine that triggers dendritic cell–mediated T helper (Th)2 inflammatory responses. TSLP is highly expressed by keratinocytes in skin lesions of patients with atopic dermatitis and is associated with dendritic cell activation in situ, suggesting that TSLP might be a master switch for allergic inflammation at the epithelial cell–dendritic cell interface. New reports now establish a direct link between TSLP expression and the pathogenesis of atopic dermatitis and asthma in vivo, and begin to reveal the molecular mechanisms underlying TSLP-induced allergic inflammation.

Thymic stromal lymphopoietin (TSLP) is a novel IL-7–like cytokine, originally cloned from a murine thymic stromal cell line, that supports the growth and differentiation of B cells and the proliferation of T cells (1, 2). The TSLP receptor (TSLPR) is heterodimeric, consisting of the IL-7R- α chain and a common γ receptor–like chain (TSLPR- γ) (3–6).

Human TSLP and TSLPR were cloned in 2001 by computational analyses of human genomic data (7, 8). The early human studies were initially frustrating because human recombinant TSLP (hTSLP), unlike mouse TSLP, did not support the development or activation of B and T cells. Surprisingly, our group found that hTSLP instead potentially activated immature CD11c⁺ myeloid dendritic cells (mDCs) (7, 9). TSLP-activated DCs induced robust proliferation of naive allogeneic CD4⁺ T cells, which subsequently differentiated into Th2 cells that produced the allergy-promoting cytokines IL-4, IL-5, IL-13, and TNF, but did not produce IL-10 or interferon- γ (9).

In vivo, TSLP was shown to be highly expressed by keratinocytes in atopic dermatitis lesions and its expression was associated with the migration and activation of Langerhans cells, sug-

gesting for the first time that TSLP might be an early trigger for DC-mediated allergic inflammation (9). Human TSLP was later found to be expressed by epithelial cells in peripheral mucosal-associated lymphoid tissue, where it activates mDCs to induce homeostatic proliferation of naive and memory CD4⁺ T cells in the periphery (10, 11). TSLP is also produced by Hassall's corpuscles in the human thymus, where it instructs thymic DCs to convert high affinity self-reactive T cells into CD4⁺CD25⁺Foxp3⁺ regulatory T cells (12).

In this commentary, we will review the recent progress in understanding the role of TSLP in the development of atopy and the underlying molecular mechanisms that govern this process.

TSLP-activated DCs create a Th2-permissive microenvironment

Like all stimuli that activate mDCs, including CD40L and Toll-like receptor (TLR) ligands, such as bacterial lipopolysaccharide (LPS), poly I:C, and R848, TSLP strongly up-regulates the expression of MHC class II, CD54, CD80, CD83, CD86, and DC-lamp on human mDCs. However, unlike CD40L and TLR ligands, TSLP does not stimulate mDCs to produce the Th1-polarizing cytokine IL-12 or the proinflammatory cytokines TNF, IL-1 β , and IL-6 (9). Our recent gene expression analyses of TSLP-activated DCs confirm and extend this finding by

showing that TSLP does not induce the expression of mRNA encoding the IL-12 family members IL-12, IL-23, and IL-27, nor that of mRNA encoding the type I IFNs—all cytokines that induce Th1 differentiation (13). Interestingly, TSLP treatment caused mDCs to produce large amounts of the chemokines IL-8 and eotaxin-2, which attract neutrophils and eosinophils, as well as TARC and MDC, which attract Th2 cells (unpublished data). We suggest that the inability of TSLP to induce the production of Th1-polarizing cytokines by mDCs is one of the most important features of TSLP-activated DCs, and helps these cells create a Th2-permissive microenvironment. The molecular mechanisms underlying TSLP's ability to promote mDC maturation without inducing the production of Th1-polarizing cytokines are unknown. TSLP appears to activate a unique signaling pathway in mDCs that is independent of the transcription factor NF- κ B and the TLR adaptor protein MyD88, both of which are required for the response to Th1-promoting stimuli. This hypothesis is supported by the fact that TSLP activates STAT5 in myeloid cells (7, 14) although the signaling molecules that function upstream and downstream of STAT5 in this pathway are currently unknown. In contrast, neither TLR ligands nor CD40L appear to activate STAT5 in mDCs.

TSLP-DCs induce inflammatory Th2 cells

In most immunology textbooks and literature, Th2 cells are defined as CD4⁺ T cells that produce IL-4, IL-5, IL-13, and IL-10, and Th1 cells such as CD4⁺ T cells that produce IFN- γ and sometimes TNF. When TSLP-DCs are used to stimulate naive allogeneic CD4⁺ T cells in vitro, they induce a unique type of Th2 cell that produces the classical Th2 cytokines IL-4, IL-5, and IL-13,

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and large amounts of TNF, but little or no IL-10 (9). Although not typically considered a Th2 cytokine, TNF is prominent in asthmatic airways, and genotypes that correlate with increased TNF secretion are associated with an increased risk of asthma (15, 16), suggesting that TNF plays an important role in the development of asthma and allergic inflammation.

In addition to inducing the production of Th2 cytokines and TNF, CD4⁺ T cells activated by TSLP-stimulated DCs produce decreased levels of IL-10 and IFN- γ , two cytokines known to down-regulate Th2 cell inflammation (17). IL-10, although initially classified as a Th2 cytokine, counteracts inflammation and is produced at decreased levels in bronchoalveolar lavage fluid from atopic patients compared with normal subjects (18). In addition, recent studies showed that DC- or T cell-derived IL-10 prevents airway hypersensitivity after allergen exposure (19, 20).

Because of their unique profile of cytokine production, we propose that Th2 cells induced by TSLP-activated DCs be called inflammatory Th2 cells. The pathogenic T cells involved in allergic diseases such as atopic dermatitis and asthma are likely to be inflammatory Th2 cells. Conventional Th2 cells that produce IL-4, IL-5, IL-13, and IL-10, but little TNF may not be involved in promoting allergic diseases but are induced in many circumstances, including when antigen-presenting cells or T cells are treated with immunosuppressive drugs, and when T cells are triggered by low affinity TCR ligands (21–23).

OX40 ligand promotes the differentiation of inflammatory Th2 cells

In an attempt to identify the molecular mechanism by which TSLP-activated DCs induce naive CD4⁺ T cells to differentiate into TNF-producing inflammatory Th2 cells, our group performed gene expression analysis on immature human mDCs that were either resting or were activated by TSLP, poly I:C, or CD40L. This analysis, reported in a recent issue of *JEM*, showed that only

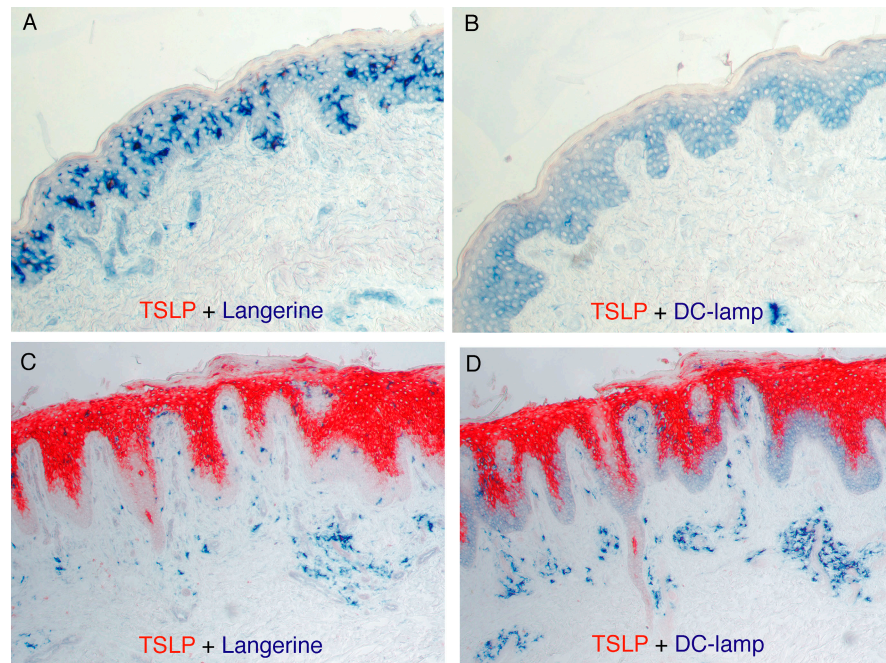


Figure 1. TSLP expression in atopic dermatitis associates with Langerhan's cell migration and activation. Normal skin contains Langerin⁺ langerhan's cells (blue) in the epidermis (a and c) and DC-lamp⁺ DCs (blue) in the epidermis and dermis (b and d). TSLP (red) is not expressed in normal skin (a and b). However, skin lesions from patients with atopic dermatitis express high levels of TSLP (c), which is associated with the migration of Langerhan's cells from the epidermis to the dermis (d). Reprinted with permission from *Nature Immunology* (Soumelis V., P.A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, et al. 2002. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat. Immunol.* 3:673–680).

TSLP induces human mDCs to express the TNF superfamily protein OX40L at both the mRNA and protein level (13).

The expression of OX40L by TSLP-DCs was important for the induction of inflammatory Th2 cells, as blocking OX40L with a neutralizing antibody inhibited the production of Th2 cytokines and TNF, and enhanced the production of IL-10, by the CD4⁺ T cells. Consistent with these results, we found that treating naive T cells with recombinant OX40L promoted the production of TNF but inhibited the production of IL-10. In other words, signals triggered by OX40L induced the generation of inflammatory Th2 cells.

OX40L-induced inflammatory Th2 cell differentiation depended on the absence of IL-12, as OX40L lost the ability to trigger inflammatory Th2 cell differentiation in the presence of IL-12. The ability of OX40L to trigger Th2 cell development was independent of IL-4, although the IL-4 that was produced by

the developing Th2 cells synergized with the OX40L-derived signals to further promote Th2 cell development. We thus conclude that TSLP-activated DCs create a Th2-permissive microenvironment by up-regulating OX40L without inducing the production of Th1-polarizing cytokines. The dominance of IL-12 over OX40L may provide a molecular explanation for the hygiene theory, which proposes that microbial infections that trigger Th1 responses may decrease the subsequent development of Th2-driven atopy.

The association of TSLP with atopic dermatitis and asthma

Early studies showed that TSLP mRNA was highly expressed by human primary skin keratinocytes, bronchial epithelial cells, smooth muscle cells, and lung fibroblasts, but not by most hematopoietic cells, including B cells, T cells, NK cells, granulocytes, macrophages, monocytes, and DCs. Interestingly, mast cells

activated by IgE receptor cross-linking expressed high levels of TSLP, suggesting an additional cell type that may help trigger allergic inflammation. TSLP protein, examined by immunohistology on cryopreserved tissue sections, is undetectable in normal skin or nonlesional skin in patients with atopic dermatitis, but is highly expressed in acute and chronic atopic dermatitis lesions (9). TSLP is expressed mainly in keratinocytes of the apical layers of the epidermis, suggesting that TSLP production is a feature of fully differentiated keratinocytes (Fig. 1). TSLP was not found in skin lesions from patients with nickel-induced contact dermatitis or disseminated lupus erythematosus. Interestingly, TSLP expression in patients with atopic dermatitis was associated with Langerhans cell migration and activation in situ (Fig. 1), suggesting that TSLP may contribute directly to the activation of these cells, which could then migrate into the draining lymph nodes and prime allergen-specific Th2 responses (9).

A more recent study showed by in situ hybridization that TSLP expression was increased in asthmatic airways and correlated with both the expression of Th2-attracting chemokines and with disease severity (24), providing the first link between TSLP and human asthma.

TSLP triggers atopic dermatitis and asthma in mice

Our own attempts to demonstrate the function of TSLP in mice were unsuccessful, leading us to believe that TSLP may function differently in humans and mice. But reports from three laboratories now reveal that TSLP and TSLPR also play a critical role in the initiation of allergic diseases in mice.

In a recent issue of *JEM*, Yoo et al. demonstrated that mice engineered to overexpress TSLP in the skin developed atopic dermatitis characterized by eczematous skin lesions containing inflammatory cell infiltrates, a dramatic increase in circulating Th2 cells and elevated serum IgE (25). This study also suggested that TSLP may directly activate DCs in mice. In another study, Li et al. reported the surprising finding that selective ablation of retinoid X receptors (RXRs) in

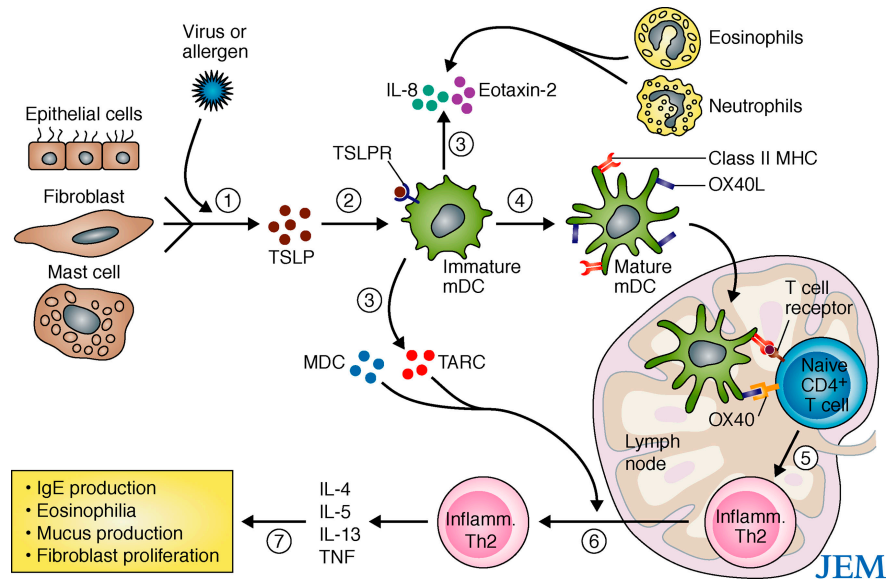


Figure 2. Pathophysiology of TSLP in allergic inflammation. Insults from allergens or viruses trigger mucosal epithelial cells or skin cells (keratinocytes, fibroblasts, and mast cells) to produce TSLP (step 1). TSLP activates immature DCs (step 2). TSLP-activated DCs produce the chemokines IL-8 and eotaxin-2, which attract neutrophils and eosinophils, as well as TARC and MDC, which attract Th2 cells (step 3). TSLP-activated mDCs mature and migrate into the draining lymph nodes (step 4). TSLP-activated DCs also express OX40L, which triggers the differentiation of allergen-specific naive CD4⁺ T cells to inflammatory Th2 cells that produce IL-4, IL-5, IL-13, and TNF but not IL-10 (step 5). Inflammatory Th2 cells then migrate back to the site of inflammation because of the local production of TARC and MDC (step 6). The Th2 cytokines IL-4, IL-5, IL-13, and TNF, produced by the inflammatory Th2 cells, initiate allergic inflammation by triggering IgE production, eosinophilia, and mucus production (step 7).

epidermal keratinocytes triggered atopic dermatitis in mice (26). The authors of that study noted that TSLP expression was rapidly induced in skin keratinocytes that lacked RXRs, likely contributing to the development of disease. This group confirmed the finding that transgenic mice overexpressing TSLP in the skin developed atopic dermatitis, thus solidifying the link between TSLP and the development of atopic dermatitis.

Two recent studies also formally established a critical role of TSLP in the initiation of asthma in vivo. Zhou et al. showed that lung-specific expression of a TSLP transgene induced allergic airway inflammation (asthma) characterized by a massive infiltration of leukocytes (including Th2 cells), goblet cell hyperplasia, and subepithelial fibrosis, as well as increased serum IgE levels (27). In contrast, mice lacking TSLPR failed to develop asthma in response to inhaled antigens (27, 28). Together, these studies demonstrate that TSLP is required

for the initiation of allergic airway inflammation in mice.

Conclusion and future perspective

We now know that TSLP is highly expressed by skin keratinocytes and airway epithelial cells during allergic inflammation (Fig. 2), but how TSLP expression is triggered in these cells—by allergen exposure or virus infection—remains unclear. As the expression of RXRs in skin keratinocytes may actively suppress TSLP production under normal physiological conditions, further studies on the regulation of these receptors may provide important clues as to how allergen or viral infection triggers TSLP production.

TSLP instructs mDCs to induce inflammatory Th2 cells in two ways (Fig. 2). First, TSLP induces DC maturation without driving the production of the Th1-polarizing cytokine IL-12, thus creating a Th2-permissive microenvironment. Second, TSLP induces the expression of OX40L on DCs, which directly

triggers the differentiation of inflammatory Th2 cells. The signaling pathway that is triggered by TSLP and leads to this unique Th2 phenotype is unknown, but it appears to involve STAT5 activation, independent of the classical NF- κ B and myD88 signaling pathways.

OX40L signaling has several important features. It triggers Th2 polarization independent of IL-4, promotes TNF production, and inhibits IL-10 production by the developing Th2 cells, but only in the absence of IL-12. In the presence of IL-12, OX40L signaling instead promotes the development of Th1 cells that, like inflammatory Th2 cells, produce TNF, but not IL-10. This finding may help explain why blocking OX40–OX40L interaction reduces the severity of Th1-mediated autoimmune diseases (29)—the reason some immunologists are reluctant to accept OX40L as a Th2 polarizing factor. We now believe that this inhibition of Th1-induced pathology is caused by the increased production of the immunosuppressive cytokine IL-10 and the decreased production of the inflammation-promoting cytokine TNF that results from blocking OX40–OX40L interactions.

Based on these recent studies, we propose the subdivision of Th2 cells into inflammatory Th2 cells that produce high levels of TNF but little IL-10, and conventional Th2 cells that produce little TNF but high levels of IL-10. Inflammatory Th2 cells, but not conventional Th2 cells, may be involved in allergic inflammatory diseases.

Our initial finding that epithelial cell-derived TSLP triggers DC-mediated inflammatory Th2 responses in humans, together with the exiting *in vivo* studies reported in the last few months, suggest that TSLP represents a master switch of allergic inflammation at the epithelial cell–DC interface. TSLP should therefore be considered as a target for immunological intervention in the treatment of allergic diseases.

REFERENCES

1. Friend, S.L., S. Hosier, A. Nelson, D. Foxworthe, D.E. Williams, and A. Farr. 1994. A thymic stromal cell line supports *in vitro* development of surface IgM+ B cells and produces a novel growth factor affecting B and T lineage cells. *Exp. Hematol.* 22:321–328.
2. Sims, J.E., D.E. Williams, P.J. Morrissey, K. Garka, D. Foxworthe, V. Price, S.L. Friend, A. Farr, M.A. Bedell, N.A. Jenkins, et al. 2000. Molecular cloning and biological characterization of a novel murine lymphoid growth factor. *J. Exp. Med.* 192:671–680.
3. Pandey, A., K. Ozaki, H. Baumann, S.D. Levin, A. Puel, A.G. Farr, S.F. Ziegler, W.J. Leonard, and H.F. Lodish. 2000. Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. *Nat. Immunol.* 1:59–64.
4. Park, L.S., U. Martin, K. Garka, B. Gliniak, J.P. Di Santo, W. Muller, D.A. Largaespada, N.G. Copeland, N.A. Jenkins, A.G. Farr, et al. 2000. Cloning of the murine thymic stromal lymphopoietin (TSLP) receptor: Formation of a functional heteromeric complex requires interleukin 7 receptor. *J. Exp. Med.* 192:659–670.
5. Fujio, K., T. Nosaka, T. Kojima, T. Kawashima, T. Yahata, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, K. Yamamoto, T. Nishimura, and T. Kitamura. 2000. Molecular cloning of a novel type 1 cytokine receptor similar to the common gamma chain. *Blood.* 95:2204–2210.
6. Hiroyama, T., A. Iwama, Y. Morita, Y. Nakamura, A. Shibuya, and H. Nakauchi. 2000. Molecular cloning and characterization of CRLM-2, a novel type I cytokine receptor preferentially expressed in hematopoietic cells. *Biochem. Biophys. Res. Commun.* 272:224–229.
7. Reche, P.A., V. Soumelis, D.M. Gorman, T. Clifford, M. Liu, M. Travis, S.M. Zurawski, J. Johnston, Y.J. Liu, H. Spits, et al. 2001. Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. *J. Immunol.* 167:336–343.
8. Tono-zuka, Y., K. Fujio, T. Sugiyama, T. Nosaka, M. Hirai, and T. Kitamura. 2001. Molecular cloning of a human novel type I cytokine receptor related to delta1/TSLPR. *Cytogenet. Cell Genet.* 93:23–25.
9. Soumelis, V., P.A. Reche, H. Kanzler, W. Yuan, G. Edward, B. Homey, M. Gilliet, S. Ho, S. Antonenko, A. Lauerma, et al. 2002. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat. Immunol.* 3:673–680.
10. Watanabe, N., S. Hanabuchi, V. Soumelis, W. Yuan, S. Ho, R. de Waal Malefyt, and Y.J. Liu. 2004. Human thymic stromal lymphopoietin promotes dendritic cell-mediated CD4+ T cell homeostatic expansion. *Nat. Immunol.* 5:426–434.
11. Rimoldi, M., M. Chieppa, V. Salucci, F. Avogadri, A. Sonzogni, G.M. Sampietro, A. Nespoli, G. Viale, P. Allavena, and M. Rescigno. 2005. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat. Immunol.* 6:507–514.
12. Watanabe, N., Y.H. Wang, H.K. Lee, T. Ito, W. Cao, and Y.J. Liu. 2005. Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature.* 436:1181–1185.
13. Ito, T., Y.H. Wang, O. Duramad, T. Hori, G.J. Delespesse, N. Watanabe, F.X. Qin, Z. Yao, W. Cao, and Y.J. Liu. 2005. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J. Exp. Med.* 202:1213–1223.
14. Isaksen, D.E., H. Baumann, B. Zhou, S. Nivollet, A.G. Farr, S.D. Levin, and S.F. Ziegler. 2002. Uncoupling of proliferation and Stat5 activation in thymic stromal lymphopoietin-mediated signal transduction. *J. Immunol.* 168:3288–3294.
15. Shah, A., M.K. Church, and S.T. Holgate. 1995. Tumour necrosis factor alpha: a potential mediator of asthma. *Clin. Exp. Allergy.* 25:1038–1044.
16. Moffatt, M.F., and W.O. Cookson. 1997. Tumour necrosis factor haplotypes and asthma. *Hum. Mol. Genet.* 6:551–554.
17. O'Garra, A. 1998. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity.* 8:275–283.
18. Borish, L., A. Aarons, J. Rumbly, P. Cvietusa, J. Negri, and S. Wenzel. 1996. Interleukin-10 regulation in normal subjects and patients with asthma. *J. Allergy Clin. Immunol.* 97:1288–1296.
19. Akbari, O., R.H. DeKruyff, and D.T. Umetsu. 2001. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat. Immunol.* 2:725–731.
20. Oh, J.W., C.M. Seroogy, E.H. Meyer, O. Akbari, G. Berry, C.G. Fathman, R.H. DeKruyff, and D.T. Umetsu. 2002. CD4 T-helper cells engineered to produce IL-10 prevent allergen-induced airway hyperactivity and inflammation. *J. Allergy Clin. Immunol.* 110:460–468.
21. de Jong, E.C., P.L. Vieira, P. Kalinski, and M.L. Kapsenberg. 1999. Corticosteroids inhibit the production of inflammatory mediators in immature monocyte-derived DC and induce the development of tolerogenic DC 3. *J. Leukoc. Biol.* 66:201–204.
22. Boonstra, A., F.J. Barrat, C. Crain, V.L. Heath, H.F. Savelkoul, and A. O'Garra. 2001. 1 α ,25-Dihydroxyvitamin D3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J. Immunol.* 167:4974–4980.
23. Constant, S.L., and K. Bottomly. 1997. Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. *Annu. Rev. Immunol.* 15:297–322.
24. Ying, S., B. O'Connor, J. Ratoff, Q. Meng, K. Mallett, D. Cousins, D. Robinson, G. Zhang, J. Zhao, T.H. Lee, and C. Corrigan. 2005. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J. Immunol.* 174:8183–8190.

25. Yoo, J., M. Omori, D. Gyarmati, B. Zhou, T. Aye, A. Brewer, M.R. Comeau, D.J. Campbell, and S.F. Ziegler. 2005. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. *J. Exp. Med.* 202:541–549.
26. Li, M., N. Messaddeq, M. Teletin, J.L. Pasquali, D. Metzger, and P. Chambon. 2005. Retinoid X receptor ablation in adult mouse keratinocytes generates an atopic dermatitis triggered by thymic stromal lymphopoietin. *Proc. Natl. Acad. Sci. USA.* 102: 14795–14800.
27. Zhou, B., M.R. Comeau, T.D. Smedt, H.D. Liggitt, M.E. Dahl, D.B. Lewis, D. Gyarmati, T. Aye, D.J. Campbell, and S.F. Ziegler. 2005. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat. Immunol.* 6:1047–1053.
28. Al-Shami, A., R. Spolski, J. Kelly, A. Keane-Myers, and W.J. Leonard. 2005. A role for TSLP in the development of inflammation in an asthma model. *J. Exp. Med.* 202:829–839.
29. Croft, M. 2003. Co-stimulatory members of the TNFR family: keys to effective T-cell immunity? *Nat. Rev. Immunol.* 3:609–620.