It was demonstrated 5 years ago that thymic stromal lymphopoietin (TSLP), a IL-7–like cytokine produced by epithelial cells, could strongly activate human myeloid dendritic cells to induce an inflammatory TH2 response characterized by high TNF-α and little IL-10 production, distinct from the regulatory TH2 responses characterized by low TNF-α and high IL-10 production. TSLP was found highly expressed by keratinocytes of skin lesions of atopic dermatitis and associated with dendritic cell activation in situ. This suggests for the first time that TSLP represents a master switch of allergic inflammation at the epithelial cell and dendritic cell interface. During the last several years, the evidence for the association of TSLP with human asthma was revealed. The direct link between TSLP expression with the pathogenesis of atopic dermatitis and asthma in vivo was demonstrated. In addition, OX40 ligand was found to be the TSLP-induced molecule on dendritic cells that triggers inflammatory TH2 differentiation in the absence of IL-12. TSLP was also demonstrated to direct the innate phase of allergic immune responses through activating mast cells. Therefore, TSLP and OX40 ligand may represent important targets for intervention of the initiation of allergic inflammatory responses. (J Allergy Clin Immunol 2007;120:238-44.)

Key words: TSLP, dendritic cells, TH2, allergy, asthma, atopic dermatitis, OX40

Allergic inflammation is the result of a complex immunologic cascade leading to the dysregulated production of TH2-derived cytokines such as IL-4, IL-5, and IL-13. Allergic inflammation in turn triggers IgE production, eosinophilia, and mucus production. Recent studies have shown that dendritic cells (DCs) play a critical role in directing the types of T-cell responses, including TH1, TH2, and TH17. The ability of DCs to induce either TH1 or TH2 responses appears to be dictated by the type of signals that the DC received at an immature stage. The link between DCs and TH1 immune responses is well defined. Specifically, microbe-derived molecules activate immature DCs through the pattern-recognition receptors, such as the Toll-like receptors (TLRs), to produce IL-12 or type 1 IFNs, which prime naive CD4+ T cells to undergo TH1 differentiation. In contrast, how DCs instruct TH2 responses is less clear. This article reviews the information supporting an important role of thymic stromal lymphopoietin (TSLP), a epithelial cell–derived IL-7–like cytokine, in regulating DCs to induce TH2 cells that are typical of atopic diseases. The important role of OX40 ligand (OX40L) expression on DCs is discussed.

**TSLP AND TSLP RECEPTOR**

Thymic stromal lymphopoietin is a 140–amino acid IL-7–like 4-helix bundle cytokine that was first isolated from a murine thymic stromal cell line and shown to support B-cell development in the absence of IL-7.
human TSLP share a poor homology of 43% amino acid identity.7-10 The human TSLP gene is localized in chromosome 5q22, not far from the gene cluster encoding for all the TH2-related cytokines: IL-4, IL-5, IL-9, and IL-13.9,10 Epithelial cells appear to be the major potential producer of TSLP in both mice and human beings.9,11 In addition, studies in the human system suggest that fibroblasts, smooth muscle cells, and mast cells all have the potential to produce TSLP.11

The TSLP receptor complex consists of a TSLP-binding chain (referred to as TSLPR) and the IL-7Rα chain9,12,13 (Fig 1). Like TSLP, human and mouse TSLPR share approximately 40% amino acid identity. By interacting with the heterodimeric receptor IL-7Rα/TSLPR, TSLP appears to initiate signal transducer and activator of transcription (STAT)–3 and STAT5 phosphorylation.9,12,13 Although TSLPR was shown to be expressed by many lymphoid and nonlymphoid tissues, the exact cell types that expressed TSLPR have not been clearly defined. Functional studies suggest that early B-cell and T-cell progenitors, peripheral CD4+ T cells, and myeloid cells may express TSLPR.7,10,12,13 (Fig 1). In the human system, extensive analyses of TSLPR mRNA in highly purified primary cells, including all human immune cell types and cell lines, show that cultured myeloid DCs (mDCs), plasmacytoid DCs after activation, and cultured mast cells have the potential to express TSLPR (Hanabuchi et al, unpublished data, February 2007). In addition, human CD4+ T cells express low levels of TSLPR after activation by anti-CD3 and anti-CD28 antibodies.14,15

**TSLP ACTIVATES mDCs TO CREATE A T\textsubscript{H}2-PERMISSIVE MICROENVIRONMENT**

Human *ex vivo*–derived mDCs rapidly express TSLPR after culture in medium or with different stimuli, and undergo maturation in response to TSLP.11 Like all stimuli that activate mDCs, including CD40 ligand (CD40L) and TLR ligands, such as bacterial LPS, polyinosinic-polyribidylic, and R848 (a TLR8 ligand), TSLP strongly upregulates 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 T\textsubscript{H}1-polarizing cytokine IL-12 or the proinflammatory cytokines TNF-α, IL-1β, and IL-6 (Table I). 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 that encodes the IL-12 family members IL-12, IL-23, and IL-27, nor the expression of mRNA that encodes the type I IFNs—all cytokines that induce T\textsubscript{H}1 differentiation.16 Interestingly, TSLP treatment causes mDCs to produce large amounts of the chemokines...
IL-8 and eotaxin-2, which attract neutrophils and eosinophils, as well as thrombin and activation regulated chemokine (TARC) and macrophage-derived chemokine (MDC), which attract Th2 cells (Table I). 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.

**TABLE I. The unique features of TSLP-DC**

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TLRL, Toll-like receptor ligand; IP-10, IFN-induced protein 10.

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 are defined as CD4+ T cells that produce IFN-γ and sometimes TNF-α. 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 classic Th2 cytokines IL-4, IL-5, and IL-13 and large amounts of TNF-α, but little or no IL-10. Although not typically considered a Th2 cytokine, TNF-α is prominent in asthmatic airways, and genotypes that correlate with increased TNF-α secretion are associated with an increased risk of asthma, suggesting that TNF-α plays an important role in the development of asthma and allergic inflammation.

In addition to inducing the production of Th2 cytokines and TNF-α, CD4+ T cells activated by TSLP-DCs produce decreased levels of IL-10 and IFN-γ, 2 cytokines known to downregulate Th1 inflammation. 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. In addition, recent studies show that DC-cell–derived or T-cell–derived IL-10 prevents airway hypersensitivity after allergen exposure.

Because of their unique profile of cytokine production, we propose that Th2 cells induced by TSLP-activated DCs be called *inflammatory Th2* cells, in contrast with the regulatory Th2 cells (Fig. 2). The pathogenic T cells involved in allergic diseases such as atopic dermatitis and asthma are likely to be inflammatory Th2 cells. Regulatory Th2 cells that produce IL-4, IL-5, IL-13, and IL-10, but little TNF-α, 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.

The classification of Th2 cells into inflammatory Th2 versus the regulatory Th2 cells may offer an explanation for why Th2-inducing parasite infection, such as Helminths infection, does not increase but instead decreases the incidence of atopy. This is because heminth infections induce the IL-10–producing regulatory Th2 cells, but not the TNF-α–producing inflammatory Th2 cells, and IL-10 may dampen adaptive immune responses to allergens.

Therefore, the classification of Th2 cells into inflammatory Th2 versus regulatory Th2 cells may also provide the experimental support for the counterregulatory model used to explain Helminths and the Th2 paradox.

**OX40L represents the original Th2 trigger from TSLP-DCs**

In an attempt to identify the molecular mechanism by which TSLP-DCs induce naive CD4+ T cells to differentiate into TNF-α–producing inflammatory Th2 cells, our group performed gene expression analysis on immature human mDCs that were either resting or activated by TSLP, poly I:C, or CD40L. This analysis showed that only TSLP induces human mDCs to express the TNF superfamily protein OX40L at both the mRNA and protein levels.

The expression of OX40L by TSLP-DCs was important for the induction of inflammatory Th2 cells, because blocking OX40L with a neutralizing antibody inhibited the production of Th2 cytokines and TNF-α 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-α but inhibited the production of IL-10. In other words, signals triggered by OX40L induced the generation of inflammatory Th2 cells. A recent study demonstrated that OX40 signaling directly induces Th2 lineage commitment by inducing nuclear factor of activated T.
cell c1, which triggers IL-4 production and then IL-4—dependent GATA-3 transcription.²⁷

THE ABILITY OF OX40L TO INDUCE TH2 DEPENDS ON A DEFAULT MECHANISM OF IL-12 ABSENCE

OX40 ligand–induced inflammatory TH2 cell differentiation depends on the absence of IL-12, because OX40L loses the ability to trigger inflammatory TH2 cell differentiation in the presence of IL-12. The ability of OX40L to trigger TH2 development is independent of IL-4, although the IL-4 that is produced by the developing TH2 cells synergizes with the OX40L-derived signals to further promote TH2 cell development.¹⁶ We thus conclude that TSLP-activated DCs create a TH2-permissive microenvironment by upregulating 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. Historically, 2 models have been proposed to explain how TH2 development is initiated: TH2 differentiation requires a positive TH2-polarizing signal (Fig 3, A), or TH2 differentiation is initiated by a default mechanism in the absence of IL-12.²⁸⁻³² (Fig 3, B). Our findings suggest that the 2 previously proposed models are not mutually exclusive and that TH2 differentiation requires a positive polarizing signal such as OX40L as well as a default mechanism (the absence of IL-12).

OX40L AND IL-4 WORK SEQUENTIALLY AND SYNERGISTICALLY IN DRIVING TH2 RESPONSES

Because anti-OX40L alone or with anti—IL-4 only partially blocks the generation of TH2 cells that produce IL-4, IL-5, and IL-13, we further investigated whether the combination of anti-OX40L and anti—IL-4 antibodies would completely block the generation of TH2 cells induced by TSLP-DCs. Indeed, we found the synergistic effect of anti-OX40L and anti—IL-4 and the combination of both almost completely switched a TH2 response to a TH1 response. Because TSLP-DCs do not produce IL-4, this experiment suggests that whereas OX40L represents the original TH2-polarizing signal from TSLP-DCs, IL-4 represents a critical autocrine stabilizer and enhancer of the developing TH2 cells. Thus, OX40L and IL-4 work
synergistically and sequentially in driving Th2 responses in T cells. 

**THE ASSOCIATION OF TSLP WITH ATOPIC DERMATITIS AND ASTHMA**

Early studies showed that TSLP mRNA is 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, natural killer cells, granulocytes, macrophages, monocytes, or 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 atop dermatitis but is highly expressed in acute and chronic atopic dermatitis lesions. TSLP is expressed mainly in keratinocytes of the apical layers of the epidermis, suggesting that TSLP production is a feature of fully differentiated keratinocytes. TSLP is not found in skin lesions from patients with nickel-induced contact dermatitis or disseminated lupus erythematosus. Interestingly, TSLP expression in patients with atopic dermatitis is associated with Langerhans cell migration and activation in situ, suggesting that TSLP production may contribute directly to the activation of these cells, which could then migrate into the draining lymph nodes and prime allergen-specific Th1 responses.

A recent study showed by in situ hybridization that TSLP expression is increased in asthmatic airways and correlates with both the expression of Th2-attracting chemokines and with disease severity, providing the first link between TSLP and human asthma. A critical role of TSLP in the development of allergic diseases has been shown in vivo by many groups. Yoo et al demonstrated that mice engineered to overexpress TSLP in the skin develop atop dermatitis characterized by eczematous skin lesions containing inflammatory cell infiltrates, a dramatic increase in circulating Th2 cells, and elevated serum IgE. 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 epidermal keratinocytes trigger atop dermatitis in mice. The authors of that study noted that TSLP expression is rapidly induced in skin keratinocytes that lack RXRs, likely contributing to the development of disease. This group confirmed the finding that transgenic mice overexpressing TSLP in the skin develop atop dermatitis, thus solidifying the link between TSLP and the development of atop dermatitis.

Two recent studies also formally establish 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) is characterized by a massive infiltration of leukocytes (including Th2 cells), goblet cell hyperplasia, and subepithelial fibrosis, as well as by increased serum IgE levels. By contrast, mice lacking TSLPR failed to develop asthma in response to inhaled antigen.

**TSLP COSTIMULATES MAST CELLS TO PRODUCE INFLAMMATORY AND TH2 CYTOKINES**

Recent studies showed that overexpression of TSLP in mice lacking Th1 cells (Th1 cell receptor β−/−) recombination activating gene (RAG−/−) triggered moderate bronchial or cutaneous allergic inflammation, suggesting that TSLP may directly activate effector cells of the innate immune system. Allakhverdi et al demonstrated that human TSLP, synergistically with IL-1 and TNF-α, stimulates mast cells to produce high levels of the Th2 cytokines IL-5 and IL-13, as well as proinflammatory cytokines GM-CSF and IL-6. This study suggests that TSLP directly activates mast cells and DCs to initiate the innate phase of allergic immune responses, followed by TSLP-activated DCs to initiate the T-cell–mediated adaptive phase of allergic immune responses.
SUMMARY AND FUTURE PERSPECTIVES

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

Thymic stroma lymphopoietin activates mast cells and mDCs to initiate the innate phase of allergic inflammatory responses. TSLP also instructs mDCs to initiate the adaptive phase of allergic immune responses. TSLP-activated mDCs express OX40L, which triggers the differentiation of allergen-specific naive CD4 \(^+\) T cells to inflammatory T\(_{H2}\) cells that produce IL-4, IL-5, IL-13, and TNF-\(\alpha\) but not IL-10. Inflammatory T\(_{H2}\) cells then migrate back to the site of inflammation because of the local production of TARC and MDC. The T\(_{H2}\) cytokines IL-4, IL-5, IL-13, and TNF-\(\alpha\), produced by the inflammatory T\(_{H2}\) cells, initiate allergic inflammation by triggering IgE production, eosinophilia, and mucus production.

FIG 6. 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. TSLP initiates the innate phase of allergic immune responses by activating immature DCs to produce the chemokines IL-8, eotaxin-2, and the T\(_{H2}\) attracting chemokine TARC and MDC and by costimulating mast cells to produce IL-5 and IL-13, as well as GM-CSF and IL-6. TSLP-activated mDCs mature and migrate into the draining lymph nodes to initiate the adaptive phase of allergic immune responses. TSLP-activated mDCs, now IL-12-producing, promote the differentiation of allergen-specific naive CD4 \(^+\) T cells to inflammatory T\(_{H2}\) cells that produce IL-4, IL-5, IL-13, and TNF-\(\alpha\) but not IL-10. Inflammatory T\(_{H2}\) cells then migrate back to the site of inflammation because of the local production of TARC and MDC. The T\(_{H2}\) cytokines IL-4, IL-5, IL-13, and TNF-\(\alpha\), produced by the inflammatory T\(_{H2}\) cells, initiate allergic inflammation by triggering IgE production, eosinophilia, and mucus production.

I would like to thank Drs Vassili Soumelis, Tomoki Ito, Yui-Hsi Wang, Norihiko Watanabe, Shino Hanabuchi, and Wei Cao for their critical contributions and the National Institute of Allergy and Infectious Diseases/National Institutes of Health, Sandler Foundation, Dana Foundation, and University of Texas MD Anderson Cancer Center for their support.
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