



Mini Review

Thymic stromal lymphopoietin programs the “allergy code” in dendritic cells

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Thymic stromal lymphopoietin (TSLP) is recently implicated as a key molecule for initiating allergic inflammation at the epithelial cell-dendritic cell (DC) interface. TSLP is produced predominantly by epithelial cells, and induces the production of multiple chemokines and cytokines by several innate cell types such as DCs, mast cells, eosinophils, and NKT cells, contributing to the innate phase of allergic inflammation. However, its function is more prominent in the DC-mediated adaptive phase of allergic inflammation. TSLP-activated myeloid DCs (mDCs) can promote naïve CD4⁺ T cells to differentiate into a Th2 phenotype. We analyzed the signal transduction mechanisms of TSLP in human primary mDCs and found that it potently transduces a unique Th2-inducing compound signal in DCs. Whereas activation of nuclear factor κ B (predominantly p50) drives DCs to produce OX40L to induce Th2 differentiation, the activation of signal transducer and activator of transcription 6 (STAT6) triggers DCs to secrete chemokines necessary for the recruitment of Th2 cells. In addition, TSLP signaling limits the activation of STAT4 and interferon regulatory factor 8 (IRF8), which are essential factors for the production of the Th1-polarizing cytokine interleukin-12. Because TSLP can be a rational therapeutic target for the treatment of allergic disorders, elucidating the mechanisms that regulate TSLP expression and the effects of TSLP on orchestrating the immune response toward a Th2 phenotype is essential for developing anti-TSLP therapy.

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Introduction

Our immune system can mount effective immune responses to invading pathogens by detecting their chemical nature. Among the many immune cell types, dendritic cells (DCs) are considered the most effective cell population that

induces distinct immune responses to different pathogens, which is often called the “functional plasticity” of DCs¹⁻³⁾. For instance, DCs induce interferon (IFN)- γ -secreting T helper type 1 (Th1)-type cells with high cytotoxicity in response to viral or intracellular bacterial infection, whereas

the same cells induce Th2-type cells that secrete interleukin (IL)-4, IL-5, and IL-13, which are required for IgE induction and eosinophil recruitment, in response to parasite infection. Allergy is a state in which the host overreacts to otherwise innocuous antigens (allergens) by inducing Th2-type inflammation. Bronchial asthma and atopic dermatitis are the representative allergic disorders. Although the precise molecular mechanisms by which DCs induce Th2-type inflammation upon recognition of allergens remain elusive, several molecular pathways that include IL-25, IL-33, thymic stromal lymphopoietin (TSLP), eosinophil-derived neurotoxin (EDN), OX40 ligand (OX40L) and several Notch ligands have been suggested for induction of Th2-type responses⁴.

TSLP is produced mainly by damaged epithelial cells and conditions the immune system to induce Th2-type immune responses. This TSLP-mediated epithelia-immune system axis was shown to be protective against a kind of intestinal helminth infection⁵. However, if other epithelial cells such as keratinocytes and bronchial epithelial cells are part of this axis, it becomes harmful by enhancing Th2-immunity, as observed in allergic disorders. TSLP is highly expressed in the lesional epidermis of atopic dermatitis patients and lungs of bronchial asthma patients. In mice, transgenic overexpression of TSLP led to allergic inflammation and TSLP receptor deficiency conferred a resistant phenotype in several allergy models⁶. Furthermore, multiple large-scale genetic association studies have indicated that human TSLP gene is one of the most common loci susceptible to bronchial asthma⁷. These findings collectively suggest that TSLP is critically involved in the pathogenesis of allergic inflammation *in vivo*.

TSLP and TSLP receptor

TSLP is an IL-7-like four-helix bundle cytokine that was first isolated from a mouse thymic stromal cell line and shown to support B-cell development in the absence of IL-7⁸⁻⁹. The human TSLP (hTSLP) was isolated using a database search method¹⁰⁻¹¹, and its gene was mapped to chromosome 5q22.1, which is near the Th2 cytokine gene cluster loci 5q23-32¹². Although epithelial cells appear to be the major source of TSLP^{11, 13}, other cell types such as fibroblasts, smooth muscle cells, mast cells and basophils have been shown to have the potential to produce TSLP as well¹³⁻¹⁴.

The TSLP receptor is a heterodimeric receptor complex consisting of the TSLPR and the IL-7R α chains^{11,15-18} (Fig.1). The TSLPR chain is most closely related to the IL-2R γ chain

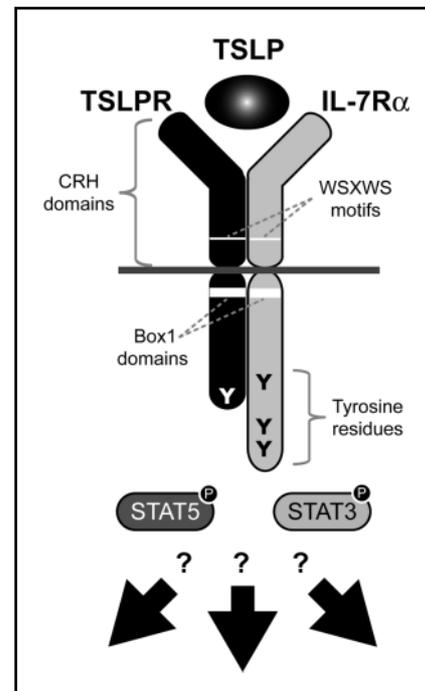
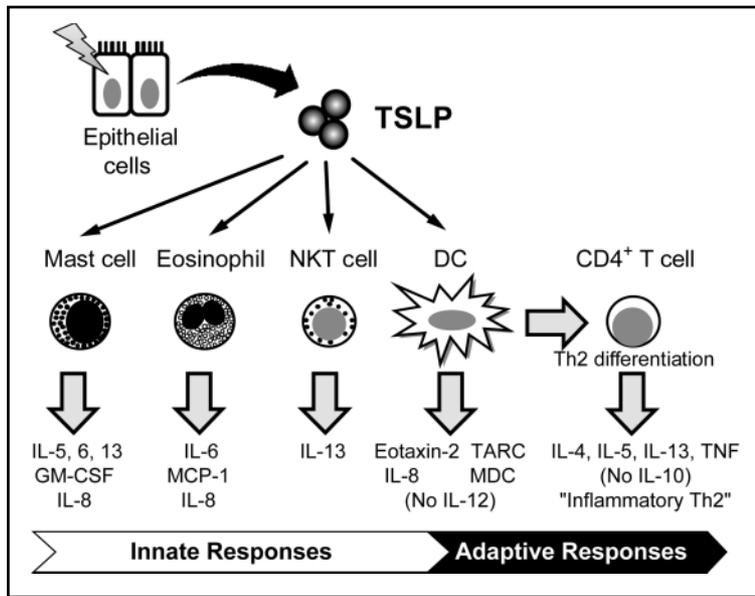


Fig.1 The TSLP receptor complex

The functional TSLP receptor complex consists of the TSLPR and IL-7R α chains. The extracellular domains of these chains comprise the cytokine recognition homology (CRH) domains and contain WSXWS motifs characteristic to most of the type I cytokine receptors. The intracellular domains of both chains contain Box1 domains and tyrosine residues that are involved in signal transduction, including STAT3 and STAT5 phosphorylation.

(γ_c). The extracellular portion of the TSLPR chain is predicted to be composed of two immunoglobulin-like folds that are likely to form a cytokine recognition homology (CRH) domain. It contains a WSXWS-like motif, a signature for type I cytokine receptor¹⁹. The intracellular portion of the TSLPR chain harbors a “box 1” motif and a single tyrosine residue, both are involved in signal transduction upon TSLP binding²⁰⁻²¹. TSLP binds to the TSLPR chain with a low affinity but does not show any affinity to the IL-7R α chain alone^{17, 18}. However, the combination of TSLPR and IL-7R α chains results in high-affinity binding to TSLP and transduces, at least, signal transducer and activator of transcription (STAT) 5 activation upon TSLP binding^{11, 15}.


Fig.2 TSLP-induced allergic immune responses

Damaged epithelial cells produce TSLP, which activates mast cells, eosinophils, natural killer T cells, and dendritic cells to produce various cytokines and chemokines to induce innate immune responses. TSLP-activated mDCs promote adaptive allergic immune responses of CD4⁺ T cells by inducing “inflammatory Th2”-type cell differentiation.

Roles of TSLP in allergic inflammation

1) TSLP induces innate allergic inflammatory responses via multiple cell types

Since the major source of TSLP is the epithelial cells, TSLP may contribute to the first-line immune response *in situ* against an epithelial break induced by allergen invasion (Fig.2). Multiple innate cell types, including DCs, mast cells, eosinophils and natural killer T (NKT) cells, have been shown to express both TSLPR and IL-7R α chains. Among these, human myeloid DCs (mDCs) show the strongest expression of the TSLPR chain, and consequently, hTSLP strongly stimulates mDCs^{11, 13, 22}. Generally, DCs can be activated or matured by many distinct classes of agents and promote T-cell proliferation and differentiation¹. Like other mDC activators, including CD40 ligand (CD40L) and Toll-like receptor ligands (TLRLs), such as lipopolysaccharide, poly(I:C) and R848, TSLP strongly upregulates the expression of major histocompatibility complex (MHC) class II, CD54, CD80, CD83, CD86, and DC-LAMP on human mDCs¹³. However, unlike CD40L and TLRLs, TSLP does not stimulate mDCs to produce the Th1-polarizing cytokines IL-12 and type I IFNs or the proinflammatory cytokines TNF, IL-1 β , and IL-6. Instead, TSLP triggers mDCs to produce large amounts of the chemokines IL-8 and eotaxin-2 (CCL24), which attract neutrophils and eosinophils, followed by production of TARC (CCL17) and MDC (CCL22), which attract Th2 cells¹³ (Table 1). Besides mDCs, hTSLP potently activates mast cells to produce the cytokines IL-5, IL-6, IL-13, and

Table 1 Features of human myeloid DCs matured by different activators

	TSLP	CD40L	TLRLs
CD80/CD86	↑	↑	↑
MHC class II	↑	↑	↑
Survival	↑	↑	↑
IL-1 α/β	-	++	++
IL-6	-	++	++
IL-12	-	++	++
IFNs	-	++	++
IP-10	-	++	++
Eotaxin-2	++	-	-
IL-8	++	++	++
TARC	++	-	+
MDC	++	+	+

GM-CSF along with the chemokines IL-8 and I-309 (CCL1) in the presence of IL-1 β , TNF, or IL-33²³⁻²⁴. Eosinophils were reported to respond to TSLP to produce IL-6, IL-8, and MCP-1 (CCL2)²⁵, and NKT cells have also been reported to produce Th2-type cytokines in response to TSLP^{26, 27}. Collectively, TSLP produced by epithelial cells rapidly triggers an innate phase of allergic inflammatory responses by activating mDCs, mast cells, eosinophils and NKT cells to produce Th2-type cytokines, chemokines, and proinflammatory cytokines. The role of TSLP in triggering an early innate phase of allergic inflammation is supported by *in vivo* studies showing that TSLP could induce skin inflammation consisting of

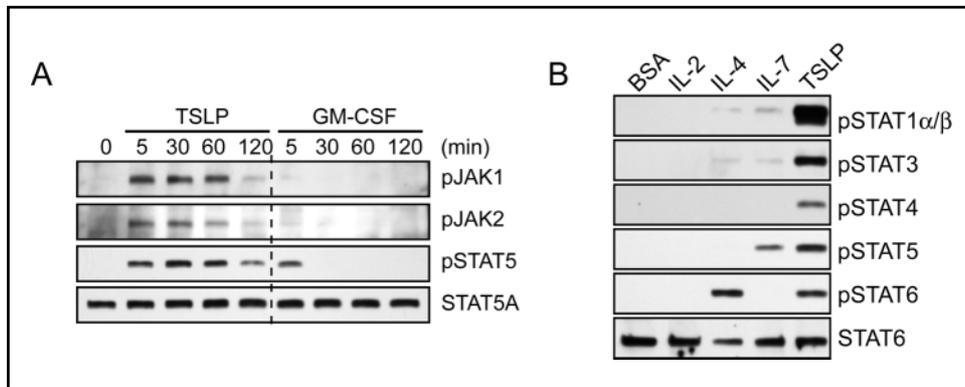


Fig.3 TSLP activates the JAK-STAT pathways in human mDCs

(A) Western blotting analysis was performed to compare the kinetics of TSLP- and GM-CSF-mediated phosphorylation of JAK1, JAK2, and STAT5 in mDCs. The STAT5A blot is shown as a loading control.

(B) Western blotting analysis was performed to compare differential STAT activation in mDCs by TSLP and the IL-2R γ chain-sharing cytokines IL-2, IL-4, and IL-7. mDCs were unstimulated (bovine serum albumin, BSA), or stimulated with 100 IU/ml of IL-2 or 50 ng/ml of IL-4, IL-7, or TSLP for 20 min at 37 °C. The STAT6 blot is shown as a loading control.

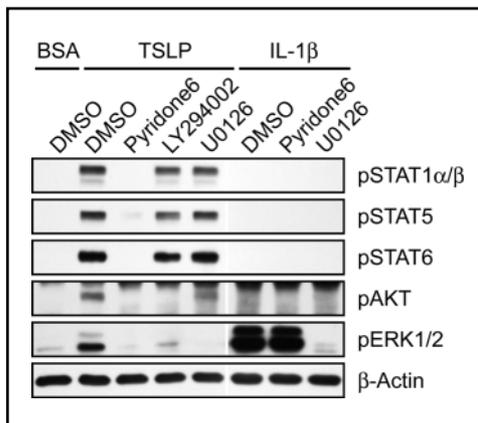


Fig.4 TSLP activates multiple signal pathways that are dependent on JAK activity

Western blotting analysis was performed to demonstrate the contributions of JAK, PI3K, and ERK to TSLP-mediated signals. mDCs were pretreated with the pan-JAK inhibitor pyridone 6, the PI3K inhibitor LY294002, the MEK (ERK) inhibitor U0126, or dimethyl sulfoxide (DMSO, 0.1%) as a vehicle control for 30 min at 37 °C and then were stimulated with 10 ng/ml of TSLP or IL-1 β or with BSA for 10 min at 37 °C. The β -actin blot is shown as a loading control.

dermal infiltrates of mast cells and eosinophils without IgE production in T cell-deficient mice²⁸) and moderate airway inflammation in B- and T-cell-deficient mice²⁹).

2) TSLP induces adaptive allergic immune responses mainly via mDCs

TSLP-activated mDCs (TSLP-DCs) not only produce multiple chemokines but also promote allogeneic CD4⁺ T cells to differentiate to a unique type of Th2 cells (Fig.2). These T cells produce the classical Th2 cytokines IL-4, IL-5, and IL-13 with large amounts of TNF but little or no IL-10. TNF expression is prominent in asthmatic airways and atopic skins^{30, 31}), and antagonism of TNF has been shown to be beneficial for asthma symptoms³²), suggesting TNF plays an important role

in the development of allergic inflammation. IL-10, initially classified as a Th2 cytokine, counteracts inflammation and was present at decreased levels in bronchoalveolar lavage fluid from atopic patients compared with normal subjects³³). Furthermore, IL-10 derived from DCs prevented airway hypersensitivity after allergen exposure³⁴). Thus, increased T cell TNF production and decreased IL-10 production by TSLP-DCs agree well with these observations for establishing allergic inflammation. Because of their unique profile of cytokine production, we propose that Th2 cells induced by TSLP-DCs be called inflammatory Th2 cells, in contrast to the conventional Th2 cells. The pathogenic T cells involved in allergic diseases, such as atopic dermatitis and bronchial asthma, are likely to be inflammatory Th2 cells.

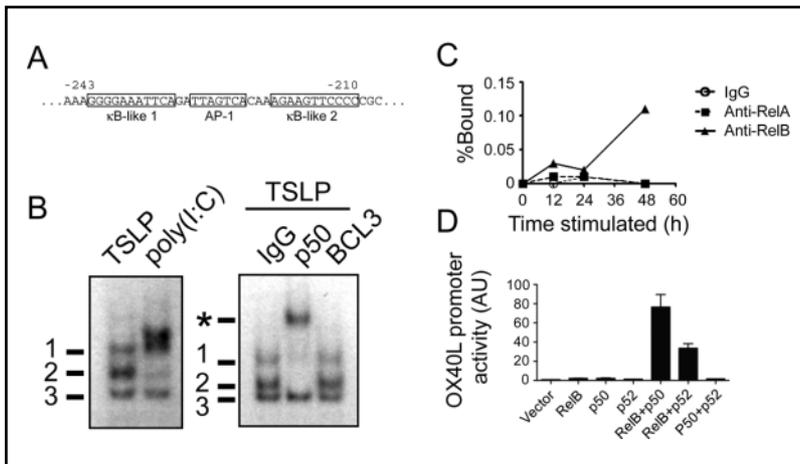


Fig.5 TSLP-mediated NF- κ B activation contributes to OX40L expression in mDCs

(A) Nucleotide sequence of the region surrounding the two potential NF- κ B binding sites within the 5'-flanking region of the OX40L gene. The numbers indicate the relative nucleotide positions from the initiator codon.

(B) *Left*, Electrophoretic mobility shift assay showing the differential binding profiles of nuclear proteins to the probe for the κ B-like sequences shown in (A) in mDCs cultured for 60 h with TSLP or poly (I:C), a TLR3 ligand. *Right*, Supershift assays were performed with antibodies against the NF- κ B p50

and BCL3 to demonstrate the composition of the nuclear proteins bound to the κ B-like probe of OX40L from lysates prepared from mDCs cultured for 60 h with TSLP. Band 2 is preferentially induced by TSLP and contains p50 but not BCL3.

(C) Chromatin immunoprecipitation assays demonstrating the kinetics of the recruitment of RelA and RelB to the OX40L promoter in TSLP-treated mDCs. Only RelB is recruited to the OX40L promoter at 48 h, when OX40L is induced by TSLP in mDCs³⁵.

(D) NF- κ B-dependent activation of the OX40L promoter in HEK 293T cells was determined by luciferase reporter assay. RelB and p50 effectively activate the OX40L promoter.

The mechanisms by which TSLP-DCs promote inflammatory Th2 cell differentiation have been explained by multiple molecular events³⁵. These events include (i) TSLP-DCs express a Th2-polarizing molecule, OX40L, (ii) TSLP-DCs do not produce Th1-polarizing cytokines, and (iii) TSLP-DCs produce Th2-recruiting chemokines. The expression of OX40L by TSLP-DCs is critical for the induction of inflammatory Th2 cells, as blocking OX40L with neutralizing antibodies inhibits the production of Th2 cytokines and TNF and enhances the production of IL-10 by the CD4⁺ T cells³⁵. The Th1-associated DC activators such as TLRs and CD40L do not induce OX40L expression by mDCs. OX40 signaling in T cells was reported to promote Th2 lineage commitment directly by inducing NFATc1, which triggers IL-4 production followed by IL-4-dependent GATA-3 transcription³⁶. Furthermore, blocking OX40L was shown to inhibit TSLP-induced asthma in mouse and nonhuman primate models of asthma³⁷. In the presence of exogenous IL-12, TSLP-DCs lost the ability to induce Th2 differentiation³⁵. We thus conclude that TSLP-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 hypothesis, which proposes that microbial infections triggering Th1 responses may hinder the subsequent development of Th2-driven atopy³⁸. The significance of inducing

Th2-recruiting chemokines by TSLP-DCs to allergic inflammation was implied by an observation that blocking TARC, one of the Th2-recruiting chemokines, *in vivo* attenuated allergen-induced airway eosinophilia and diminished the degree of airway hyper-responsiveness with a concomitant decrease in Th2 cytokine levels³⁹.

TSLP signal transduction in human DCs

Because this recent progress showed the importance of TSLP in the pathogenesis of allergic diseases, understanding the mechanism of TSLP signal transduction is critical in developing a therapeutic strategy to target its function. Upon binding to TSLP, the TSLP receptor complex generates intracellular signaling. Earlier studies demonstrated that TSLP induces STAT3 and STAT5 phosphorylation, resulting in transcription of the STAT-responsive genes, such as *CIS*^{11,15-16}. However, the precise kinase responsible for TSLP-mediated STAT phosphorylation has long remained controversial^{15-16,21}. Moreover, the pleiotropic function of TSLP in human mDCs apparently cannot be explained by activation of STAT3 and STAT5 alone, which is ubiquitous in many cytokine signaling pathways. Thus, we performed a large-scale isolation of human primary mDCs to study intracellular signaling triggered by TSLP⁴⁰. In mDCs, we found that TSLP induces robust and sustained (-1 h) phosphorylation of JAK1 and JAK2, while GM-CSF induces faint and transient (-5 min)

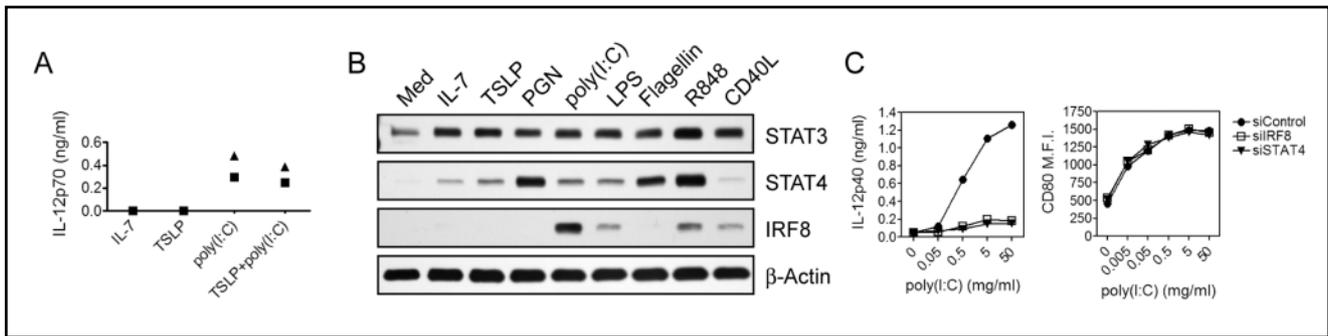


Fig.6 TSLP does not stimulate the production of IRF8 or STAT4, essential factors for the production of IL-12 by mDCs

(A) The production of IL-12p70 by mDCs cultured with IL-7, TSLP, poly(I:C), or TSLP plus poly(I:C) for 24 h was determined by ELISA. The square and triangle each represent one of two donors. TSLP is unable to inhibit poly(I:C)-induced IL-12p70 production, suggesting that TSLP is not a dominant repressor of IL-12 production.

(B) Western blotting analysis was performed to compare the differential regulation of the abundance of IRF8 and STAT4 in mDCs that were activated for 24 h by the indicated stimuli, including cytokines, TLRs and CD40L. The abundance of STAT3 is comparable among all the stimuli. The β -actin blot is shown as a loading control.

(C) mDCs transfected with small interference RNA (siRNA) were stimulated with poly(I:C) for 24 h and the concentration of IL-12p40 in the culture supernatants and the mean fluorescent intensity of cell-surface CD80, one of the DC activation markers, are depicted. Knocking down IRF8 or STAT4 deeply affects IL-12 production but not induction of CD80. Since TSLP-DCs do not upregulate the abundance of IRF8 and STAT4, they are unable to produce IL-12.

phosphorylation of JAK2 (Fig.3A). Consequently, TSLP induces broad and sustained (>2 h) phosphorylation of STAT1, STAT3, STAT4, STAT5, and STAT6 (Fig.3B). Direct STAT6 activation by TSLP seems to be the responsible mechanism for inducing TARC production⁴⁰. In addition, TSLP induces phosphorylation of AKT and the MAPKs ERK and JNK, all are sensitive to JAK inhibitors (Fig.4). TSLP also induces a slow but robust NF- κ B activation, as revealed by sustained nuclear localization of p50, p52, and RelB⁴⁰.

How is OX40L selectively upregulated by TSLP-DCs? The promoter region of OX40L has two potential NF- κ B binding elements (Fig.5A)⁴¹. We found that TSLP induces a predominant and persistent nuclear translocation of p50, an NF- κ B molecule, which is not prolonged when induced by CD40L or TLRs⁴⁰. Indeed, the OX40L promoter preferentially binds to p50 (Fig.5B). RelB but not RelA is also recruited to the OX40L promoter region at a later time point upon TSLP stimulation (Fig.5C). In a promoter-reporter assay, we also demonstrated that p50 plus RelB activates the OX40L promoter (Fig.5D). These data collectively suggest that predominant p50 activation triggered by TSLP is the determinant for upregulation of OX40L.

Why TSLP-mediated DC maturation is uncoupled from IL-12 production was the next issue because most of the DC activators, such as CD40L and TLRs, induce both DC matu-

ration and IL-12 production to induce Th1 responses¹. Unlike these other DC activators capable of inducing expression of both STAT4 and interferon regulatory factor (IRF) 8 that are required for IL-12 production, TSLP simply fails to induce protein expression of these transcription factors (Fig.6). Although the protein expression of STAT4 by TSLP is largely limited, TSLP does have the capacity to activate STAT4 as shown in Fig. 3B. Thus, perhaps IRF8 is a relatively more critical regulator of IL-12 production than STAT4 in DCs.

In conclusion, these findings demonstrate that TSLP programs human mDCs to induce Th2 responses by activating multiple signal pathways, which together work in a unique manner as an “allergy code”.

Conclusion

We summarized recent progress regarding the roles of TSLP in allergic inflammation pathogenesis and showed our recent findings on its signal transduction mechanism in human primary mDCs. Important questions concerning the spatial and temporal role of TSLP in inflammatory responses *in vivo* and whether blockade of TSLP will be efficacious in the treatment of allergic inflammatory diseases remain unanswered⁴². The production of TSLP by activated mast cells and activation of mast cells by TSLP indicate TSLP's role in the progression of allergic diseases^{13, 23, 24}. Recently,



the existence of a mutually amplifying loop between TSLP and IL-13 in allergy was suggested, illuminating the critical involvement of TSLP in both the initiation and progression of allergic diseases⁴³⁻⁴⁴). The findings that anti-TSLP therapy is beneficial in resolution of allergic symptoms in mice⁴⁵⁻⁴⁶) provide some promise that TSLP will be a potential therapeutic target for the treatment of allergic inflammatory disorders in humans.

Acknowledgements

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