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Thymic stromal lymphopoietin and the pathophysiology of atopic disease

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Abstract

Thymic stromal lymphopoietin (TSLP) is an IL-7-related cytokine expressed predominantly by barrier epithelial cells. TSLP is a potent activator of several cell types, including myeloid-derived dendritic cells, monocytes/macrophages and mast cells. Recent studies have revealed an important role for TSLP in the initiation and progression of allergic inflammatory diseases. In this review, we will discuss the role of TSLP in atopic diseases, as well as its function in immune homeostasis.

Keywords

allergy; atopy; epithelial cell; inflammation; lung; skin; Th2; TSLP

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Identification of thymic stromal lymphopoietin & its receptor complex

Thymic stromal lymphopoietin (TSLP) is a cytokine with structural and functional similarities to the hematopoietin family of cytokines. It was originally characterized as the factor responsible for the lymphoproliferative activity contained in conditioned media from a mouse thymic stromal cell line [1]. It was found to promote the development of IgM⁺ B cells, as well as costimulate developing thymocytes [1,2]. A TSLP homolog was subsequently identified in humans via sequence homology [3]. Despite sharing only 43% amino acid identity, human and mouse TSLP share key structural motifs, including the four α -helices characteristic of the hematopoietin family, six conserved cysteine residues, and multiple potential *N*-linked glycosylation motifs. More importantly, as discussed later, human and murine TSLP share a high degree of functional homology.

The TSLP receptor (TSLPR) was cloned from and detected in TSLP-responsive cells [3–5]. While it is classified as a hematopoietin receptor based on gross structural homology, there are several key differences from the canonical hematopoietin receptor structure. Most notably, it contains only a single tyrosine residue on its cytoplasmic tail and is missing the box2 motif used by other hematopoietin receptors to mediate Janus protein tyrosine kinase (JAK) signaling. In addition, the extracellular domain lacks one of the four conserved cysteine residues, and utilizes a modified WSXWS motif, indicating that the TSLPR may adopt a noncanonical extracellular folding pattern. Both murine and human TSLPR share the aforementioned structural motifs. Similar to the cytokine, the murine TSLPR shares only 39% identity with its human counterpart, yet the two appear to be functionally homologous.

The effects of TSLP on B and T cells closely resemble the activity of the hematopoietin family member IL-7 [1–3]. Consistent with these observations, it was found that the affinity of TSLPR for TSLP was greatly enhanced when paired with the α -chain of the IL-7 receptor (IL-7R α) [4,5]. Unlike IL-7, TSLP activity did not require the common γ chain (γ_c), indicating that the TSLPR/IL-7R α heterodimer forms the complete functional TSLP receptor complex [2,5]. This receptor arrangement is similar to the receptor complexes for IL-4 and IL-13; IL-4 signals through the IL-4R α / γ_c complex, whereas IL-13 signals via an IL-4R α /IL-13R complex (reviewed in [6]). However, while IL-4 can signal through both the IL-4 and IL-13 receptor complexes, IL-7 cannot signal through the TSLP receptor complex [2,3,5,7]. Thus, IL-7 and TSLP, while sharing some functional homology, mediate their effects through physically independent receptor complexes. It is interesting to note that both TSLPR and IL-7R α have multiple potential *N*-linked glycosylation sites. It has recently been shown that glycosylation of IL-7R α is required for high-affinity binding of IL-7 to its receptor complex [8]. Determining the effect that glycosylation has on TSLP signaling may thus yield additional insight into the relationships between the two cytokines.

TSLP & IL-7 play overlapping roles in lymphocyte development

The hematopoietin receptor family is known to mediate signaling activity through the JAK–STAT pathways. In particular, IL-7 is known to activate JAK1 and JAK3 via recruitment to IL-7R α and γ_c , respectively, which in turn activate STAT5 (reviewed in [9]). Like IL-7, TSLP can activate STAT5 and drive the transcription of STAT5-responsive gene targets [2–4,7,10]. TSLP has also been shown, in a human B-cell line, to activate STAT3 in addition to STAT5 [3]. Interestingly, TSLP-mediated STAT activation occurs without activating any of the known JAK family members, although inhibition of Tec kinase was able to partially abrogate the ability of TSLP to drive STAT5-dependent transcription [2,7]. Furthermore, a Src kinase inhibitor abrogated the ability of TSLP to drive cell proliferation while leaving STAT5 activation unaffected, indicating that TSLP can also induce STAT-independent activities. The sole cytoplasmic tyrosine residue of TSLPR appears to be required for this latter activity

[10]. Exactly how TSLP induces JAK-independent STAT activation, as well as the nature of TSLP's STAT-independent activity, remain open to discussion.

Despite its initial characterization as a lymphoproliferative cytokine related to IL-7, attempts to demonstrate a nonredundant physiological role for TSLP in the development of lymphocytes have been met with little success. While *in vitro* evidence points to a unique role for TSLP in promoting the maturation of pro-B cells to pre-B cells [1,2], mice lacking TSLPR have a normal B-cell compartment [11]. TSLP was found to support the growth of CD4⁺ CD8⁺ T-cell precursors [12], and TSLPR^{-/-} CD4⁺ T cells had some defects in homeostatic expansion [13], but the overall effects of TSLP on thymocyte development were, at best, modest. Indeed, it is now apparent that TSLP's important physiological activities are primarily mediated through myeloid cells rather than cells of lymphoid lineages.

Expression patterns of TSLP & TSLPR provide insight into TSLP's primary biological role

A significant breakthrough in understanding TSLP's primary biological role came from an analysing of the expression of TSLPR. In addition to the known expression in T and B cells, it was observed that human myeloid cells expressed very high levels of both IL-7R α and TSLPR. Treatment of human dendritic cells (DCs) and monocytes *in vitro* was found to potently activate the cells. Additionally, naive CD4⁺ T cells primed on these TSLP-activated DCs were found to display a distinct Th2-inflammatory cytokine profile [3,14]. Murine bone marrow-derived DCs were found to acquire a similarly activated phenotype under TSLP stimulation [15]. Soon after, it was observed that TSLP, despite being cloned from a thymic stromal cell line, was in fact expressed predominantly by epithelial cells of the skin, lung and gut. Importantly this expression pattern was significantly increased in the epithelia of patients suffering from atopic disease [14]. These observations, discussed at greater length later in this review, formed the basis of the hypothesis that TSLP is a key participant in the development of Th2-type inflammatory immune responses.

In addition to TSLP's effects on peripheral myeloid cells, it was also shown that TSLP-stimulated human thymic DCs upregulate CD80 and CD86, and are capable of driving the differentiation of FoxP3⁺ regulatory T cells in the thymus [16]. Accordingly, TSLP production in the human thymus was found to be associated with Hassall's corpuscles, and it was shown that FoxP3⁺ regulatory T cells were exclusively colocalized in these same areas in close association with activated mature DCs [16]. Although mice lack Hassall's corpuscles, further studies using various knockout mice have found that either IL-7 or TSLP capable of supporting the differentiation of regulatory T cells [17]. However, these findings have been disputed [18], suggesting that there may be aspects of TSLP's effects on regulatory T cells that are fundamentally unique to the human system.

TSLP activity is induced by NF- κ B-mediated inflammation

The discovery that TSLP is an epithelial-derived, inflammation-associated cytokine led to an interest in determining how its expression is regulated. Our group had observed that TSLP was upregulated *in vitro* in normal human bronchial epithelial cells by the inflammatory cytokines TNF- α and IL-1 β . By cloning the proximal TSLP promoter, we determined that TSLP expression is controlled by an upstream NF- κ B site, and that NF- κ B activity was both necessary and sufficient to drive TSLP transcription [19]. Indeed, treatment with Toll-like receptor (TLR) ligands, known inducers of NF- κ B signaling, also results in the production of TSLP by epithelial cells [19,20]. Further studies have demonstrated that NF- κ B-induced TSLP expression can be antagonized by the activity of the retinoid X receptor (RXR), both *in vitro* [21] and *in vivo* [21–23].

Thymic stromal lymphopoietin expression has also been shown to be regulated by RXRs *in vivo*. Conditional ablation of RXR α and RXR β in keratinocytes results in overexpression of TSLP and the subsequent development of an atopic dermatitis (AD)-like disease in mice [24]. This suggests that RXRs, in the absence of their ligand, inhibit TSLP expression in the steady state, potentially by recruiting corepressors to the TSLP promoter region. Interestingly, treatment of mice with vitamin D analogs, which can serve as ligands for RXRs when they heterodimerize with the vitamin D receptors, results in a similar AD-like disease as seen in the RXR α/β keratinocyte conditional knockout mice [22]. Taken together, these data suggest that in the absence of a ligand, RXRs inhibit TSLP transcription; however, in the presence of a ligand, such as vitamin D analogs, this transcriptional repression is released, resulting in TSLP expression. Putative RXR binding sites have been identified within the TSLP promoter region; however, it remains to be shown whether these sites are functional. In addition, it will be important to determine how RXRs mediate transcriptional regulation of TSLP in the steady state and how this inhibition is released in the presence of a ligand, whether it be by recruitment of corepressors and coactivators, as has been shown for RXRs in other contexts, or by an alternative mechanism [25].

The regulation of TSLPR is less well understood. It appears to be expressed on freshly isolated DCs and monocytes, as well as T and B cells, and can be upregulated when these cells are activated with pro-inflammatory mediators such as TNF- α and TLR ligands [3]. That these inflammatory mediators also induce TSLP expression by epithelial cells creates a situation where an inflammatory event can drive both the production of and response to TSLP, allowing the TSLP response to be efficiently propagated. Unpublished data from our lab have shown that TSLPR is also upregulated in epithelial cells in response to various inflammatory mediators; the significance of receptor expression by epithelial cells remains unclear [Miazgowicz MM, Ziegler SF, Unpublished data].

TSLP overexpression induces systemic inflammatory disease

Given the potent effects TSLP has on myeloid cells and, to a lesser extent, lymphoid cells, it was surprising that mice lacking TSLPR were overtly indistinguishable from their wild-type littermates [11]. However, given that the activity of TSLP was associated with states of inflammation, it was possible that the lack of phenotype in TSLPR^{-/-} mice reflected a primary role for TSLP under inflammatory conditions rather than during steady-state conditions. To determine the effects of elevated TSLP expression, mice overexpressing TSLP under several different promoters were created. The first of these mouse lines contained a TSLP transgene under the control of the proximal *lck* promoter, restricting its overexpression to early thymocytes. These mice developed a diverse array of symptoms, including ulcerative skin lesions of the ear, splenomegaly, a cryoglobulinemic glomerulonephritis and an eosinophilic leukocyte infiltration of the lungs [26]. The mice ultimately succumbed to the lung infiltration at approximately 7 months of age.

Contemporaneously, our group developed a mouse line that expressed TSLP under the control of the *c-fes* promoter [27], driving the overexpression of TSLP during early hematopoiesis, as well as in all myeloid cells. This resulted in profound systemic inflammation, including massive splenomegaly and lymphadenopathy, as well as a severe leukocytic infiltration of the lungs. Unlike the *lck*-TSLP mice, symptoms in the *c-fes*-TSLP mice presented early in life, with all founder mice succumbing to the inflammation prior to, or at the time of, weaning [Zhou B, Ziegler SF, Unpublished data]. These results, combined with the results from *lck*-TSLP mice, indicate that the timing and location of TSLP expression has profound effects on the immune system, and that an accurate assessment of the physiological role that TSLP plays would require a more targeted expression approach. A variety of mouse model systems have now been developed in order to address this issue, including models of TSLP overexpression in both the

skin and the lungs. The following sections will discuss how the data from these models have elucidated a crucial role for TSLP in Th2-type inflammatory diseases. Understanding the full range of roles that TSLP plays in these mice model systems is the first step towards designing rational TSLP-based therapies for corresponding human atopic diseases.

Role of TSLP in asthma pathogenesis

Asthma is a chronic inflammatory disease of the lungs. Traditionally, asthma has been defined by the presence of multiple characteristic symptoms including airway hyperresponsiveness, leukocytic infiltration of the airways and lung tissue, including significant eosinophilia, subepithelial fibrosis, mucous hyper-secretion, and dramatic increases in serum IgE. Furthermore, it has been clearly shown over the past few decades that Th2 cells and associated cytokines and chemokines are crucial for the pathogenesis of this disease. Many of the known hematopoietic lineages play prominent roles in asthma, including Th2-skewed CD4⁺ T cells, regulatory T cells, mast cells, eosinophils, natural killer (NK) T cells, basophils, alveolar macrophages, B cells and various DC populations. A significant proportion of the pathogenic roles attributed to these cells involves the production and response to various Th2-associated cytokines and chemokines, including IL-4, IL-5, IL-13, IL-9, TNF- α , eotaxin, CCL17 and CCL22 (recently reviewed in [28]).

Until recently, it was largely thought that cells of hematopoietic origin were the primary pathogenic players in asthma. Over the past 10 years or so, it has become increasingly clear (as it has with other allergic diseases including atopic dermatitis, as discussed later) that epithelial cells themselves can play a central role in governing the development of allergic disease. This largely stems from the production of cytokines such as TSLP, IL-25 and IL-33, which appear to serve as a communicative network between the epithelium and the hematopoietic cell lineages (reviewed in [29,30]). TSLP, in particular, has now been shown to be both a necessary and sufficient factor in the establishment of disease in murine models of asthma, and evidence suggests it also is likely to play a role in human disease [31,32].

TSLP expression is deterministic in asthma pathogenesis

The first evidence for an important role of TSLP in asthma came from bronchial biopsies taken from asthmatics and healthy non-asthmatic controls. The asthmatic tissue showed dramatically increased expression of TSLP mRNA by *in situ* hybridization when compared with the control tissue. Most importantly, Ying *et al.* found an inverse correlation between TSLP mRNA expression and forced expiratory volume in 1 s (FEV₁) measurements [31]. Given the strong correlation that exists between asthma severity and decreased FEV₁ [33], this was highly suggestive of a causative role for TSLP in asthma development and/or progression. Studies using animal models from our lab and others have since confirmed this hypothesis [34–36]. Mice generated to express a TSLP transgene driven by the surfactant protein C promoter, restricting TSLP overexpression to type III lung epithelium, were found to spontaneously develop airway inflammation, presenting with all of the cardinal signs of human asthma [34]. Further establishing that increased TSLP in the lung can drive asthma development, we and others have also found that direct intranasal administration of TSLP into wild-type mice can similarly drive an asthmatic-like phenotype, provided antigen is administered [35,36]. Importantly, in antigen-driven animal models of asthma, TSLP activity is also necessary for disease development. For example, in the classic ovalbumin (OVA)/Alum priming/challenge model of airway inflammation in mice, wild-type mice in this model strongly upregulate TSLP expression in the lung. By contrast, TSLPR-deficient mice fail to develop significant airway inflammation [34,37]. The previously described studies have served to elucidate a crucial role for TSLP in murine models of asthma and have suggested this may also be true of human disease. Future studies are, however, still necessary in order to determine whether this is fully generalizable to human asthma.

A series of recent studies have suggested that the role of TSLP in respiratory diseases may extend beyond asthma. In a recent study, Mou and colleagues demonstrated that allergic rhinitis patients display elevated levels of both TSLP mRNA and protein in their nasal mucosa [38]. In addition, a 2007 study by Zhang *et al.* demonstrated that human airway smooth muscle cells from chronic obstructive pulmonary disease patients have a heightened capacity to express TSLP in response to the inflammatory stimuli IL-1 β and TNF- α [39]. Cigarette smoking has long been known to be a significant risk factor in asthma development, as well as in disease exacerbations. Exposure of mice to cigarette smoke extract leads to significant increases in TSLP expression and, furthermore, intranasal treatment of mice with cigarette smoke extract alongside the experimental allergen OVA leads to a Th2-characterized inflammation of the lungs, which can be blocked by cotreatment with a TSLP-neutralizing antibody [40]. Taken together, these studies highlight the key role played by TSLP not only in asthma but also in other respiratory diseases. However, a great deal of future research is needed to establish the role of TSLP under these nonasthmatic conditions.

Cellular & molecular mediators of TSLP-driven asthma

Although the true physiologic role of TSLP in the lung has yet to be fully elucidated, a number of recent studies have fleshed out the cellular- and cytokine-driven pathways extending from TSLP to the asthma phenotype. As with human asthma and other murine models of asthma, TSLP-driven airway inflammation is largely mediated by activities of cells of the adaptive immune system. RAG^{-/-} mice, deficient in T and B lymphocytes, are highly resistant to disease development in either the surfactant protein C (SPC)-TSLP or intranasal TSLP models described previously, as well as in the OVA/Alum model. The role of lymphocytes appears to be largely a requirement for CD4⁺ T cells and not B cells, although B cells may contribute to the magnitude of the response. Interestingly, in contrast to the spontaneous disease that develops in SPC-TSLP mice, intranasal administration of TSLP requires the co-administration of a foreign antigen in order for disease to develop [36]. Furthermore, SPC-TSLP mice whose T cells are restricted to a single specificity through the expression of a T-cell receptor transgene (SPC-TSLP X DO11.10) fail to develop airway inflammation in the absence of OVA [Headly MB, Ziegler SF, Unpublished data]. This suggests that a primary mechanism of TSLP-induced asthma may be to enable pathogenic adaptive immunity against environmental allergens to which the individual would otherwise be tolerant.

In keeping with this model, treatment of human myeloid DCs with TSLP (TSLP-DC) results in the upregulation of a variety of molecules involved in costimulation and antigen presentation, including CD80, CD86, OX40L and MHC class II [14,41,42]. TSLP-DCs also produce the Th2-associated chemokines CCL17 and CCL22, which are important for recruiting Th2-polarized CD4⁺ T cells to sites of inflammation [3,14]. TSLP-DCs induce the differentiation inflammatory Th2 cells, which produce IL-4, IL-5, IL-13 and TNF- α , as opposed to the conventional Th2 cell, which has been characterized to produce high levels of IL-10 rather than TNF- α [14]. IL-10 is a regulatory cytokine that is able to inhibit inflammatory responses, including allergic asthma, whereas TNF- α is an inflammatory cytokine present at high levels in asthmatic lungs and in the lesional skin of AD patients [43]. In the mouse, the role of TSLP signaling in DCs is less clear. One report showed that mouse DCs treated with TSLP do not appear to directly prime T cells towards a Th2 phenotype *in vitro* but negatively regulate IFN- γ production [37]. However, our lab has data demonstrating that TSLP-treated DCs can lead to an increase in IL-4-producing cells under Th2 culture conditions [Kitajima M, Ziegler SF, Unpublished data]. This suggests that TSLP may simultaneously promote Th2 responses and repress Th1 responses. This hypothesis is supported by studies investigating the role of TSLP in *Trichuris muris* infection in the gut [44].

Mast cells [45–48], basophils [49], eosinophils [50] and macrophages [51] have been shown in various contexts to interact with the TSLP inflammatory axis, but to date the role these cells may play in TSLP-mediated asthma has not been evaluated. Allakhverdi *et al.* recently showed that human CD34⁺ hematopoietic progenitor cells (which peripherally differentiate into mast cells, eosinophils and basophils) express functional TSLPR and respond to TSLP in combination with the pro-allergic cytokine IL-33 by producing large amounts of Th2 cytokines [52]. The role of this increased cytokine production in asthma pathogenesis has not been directly evaluated. A clear role has been defined, however, for invariant chain NKT (iNKT) cells in murine systems, although this has not been confirmed in humans. *In vitro* studies have shown that TSLP treatment results in a marked increase in IL-13 production by iNKT cells [53]. In addition, iNKT-deficient SPC-TSLP mice display dramatically reduced AHR, but intact pulmonary eosinophilia, serum IgE and IL-4 production relative to iNKT-sufficient animals. Consistent with the *in vitro* data, these mice display a significant reduction in lung IL-13 [53].

On a molecular level, data indicate that TSLP sits at the apex of the Th2 cytokine axis. In both SPC-TSLP mice and mice receiving intranasal TSLP, significant increases in the Th2 cytokines IL-4, IL-13 and IL-5 are observed [34,35,54]. Furthermore, crossing SPC-TSLP mice with either IL-4^{-/-} mice or STAT6^{-/-} mice (lacking the ability to signal through either IL-4 or IL-13) significantly reduces disease development in the case of IL-4^{-/-} deficiency, and completely protects against disease in the STAT6^{-/-} mice. This suggests that both IL-4 and IL-13 are key to the development of TSLP-mediated airway inflammation [54]. The NKT cell data described previously further indicate that Th2 cytokines may play distinct roles in the overall disease phenotype [53]. OX40L appears to be at least one of the links between TSLP and Th2 cytokine production. TSLP-treated human DCs strongly upregulate OX40L, and antibody-mediated blockade of this receptor negatively impacts Th2 priming of T cells by TSLP-treated DCs *in vitro* [42]. In addition, blockade of OX40L in mice receiving intranasal TSLP leads to reduced disease severity, as well as reductions in the levels of IL-4, IL-13 and IL-15 in the lung [35].

Asthma-associated stimuli can induce TSLP expression

It has long been known that severe childhood respiratory virus infection correlates strongly with subsequent asthma development. To date, only a small number of studies have focused on the possibility that TSLP expression may be a factor in this predisposition. In 2007, Kato *et al.* found that either the viral mimetic polyinosinic:polycytidylic acid (polyI:C) or influenza virus infection can induce TSLP expression by normal human bronchial epithelium in a TLR3-dependent manner [20]. Recent studies from our laboratory and others have also found that viruses of the paramyxovirus family, Sendai virus and respiratory syncytial virus, which have been shown in murine and human systems to strongly associate with asthma development, can potently induce TSLP expression in both murine and human bronchial epithelium [Headley MB, Lee HC, Ziegler SF, Unpublished data] [55]. In addition to viral infection, cigarette smoking is commonly associated with asthma risk and, as discussed previously, at least in the murine system, cigarette smoke extract can similarly induce TSLP expression by airway epithelial cells and favor allergen sensitization in a TSLP-dependent fashion [40]. More research needs to be focused on this area in order to elucidate whether TSLP can act to facilitate asthma development downstream of these various signals. Importantly, no studies to date have evaluated TSLP function in the immune response to classic human allergens, such as cockroach allergen, Derp1 or FelD1, all of which favor allergy at least in part through intrinsic proteolytic activities.

Genetic links between TSLP & asthma

There is mounting evidence that genetic differences in genes encompassing the TSLP signaling pathway may result in an increased risk of allergy and asthma development. Hunninghake and colleagues identified a female-specific TSLP variant allele in a Costa Rican cohort that is negatively associated with serum-test positivity for cockroach allergen-specific IgE [56]. In a separate study, a significant association was found between two separate alleles of the *IL-7Ra* gene and patients positive for inhalation allergy [57]. Most recently, a genome-wide association scan looking at eosinophilia in asthma and myocardial infarction in an Icelandic population identified a positive correlation between asthmatics and a single-nucleotide polymorphism (SNP) in significant linkage disequilibrium with the *TSLP* gene [58]. What may be the most compelling genetic evidence to date comes from a recent small cohort study that identified a SNP in the *TSLP* gene that results in the formation of a functional activator protein-1 site. This allele leads to increased TSLP promoter activity in response to polyI:C [59]. These data suggest the possibility that genetic variation in the TSLP gene and/or genes associated with TSLP signaling can lead to modifications in both TSLP expression in response to inflammatory stimuli as well as susceptibility to asthma and related allergic disorders.

TSLP in skin inflammation & allergy

In addition to asthma, a strong association exists between TSLP and inflammatory diseases in the skin. The skin is a complex organ, containing a variety of layers made up of diverse populations of hematopoietic and epithelial cells, both of which are integrally involved in orchestrating an immune response to pathogens and innocuous environmental antigens [30]. AD is a chronic allergic inflammatory disorder of the skin, which occurs due to a combination of genetic susceptibility and environmental/antigenic insult, such as exposure to allergens. Until recently, it was unclear which immune mediators were responsible for the sensitization to allergens, the initial step in allergy development. Epithelial cells appear to play a more significant role in shaping the immune response at barrier surfaces than previously thought. It is now clear that TSLP, most likely derived from keratinocytes, acts on skin-resident leukocytes, namely mast cells and DCs [14,45]. This results in local inflammation due to the activation of cells of the innate immune system and sensitization to allergens via the activation of a Th2 response by TSLP-programmed DCs [60]. The following will review what is known about TSLP and the role it plays in human AD and mouse models of AD-like disease.

TSLP & skin inflammation in humans

Atopic disease has been linked to genetic polymorphisms in a region of the genome called the epidermal differentiation complex, and currently there are no reported polymorphisms in or around the *TSLP* gene that are linked to AD [43]. The epidermal differentiation complex is a region of the genome that contains genes whose protein products are involved in barrier function and development. For example, filaggrin is a structural protein involved in multiple steps of epithelial differentiation and barrier function. Mutations in the filaggrin gene result in altered barrier function and a significant predisposition for developing AD [61,62].

A role for TSLP in the development of AD was first hypothesized when it was found in high levels of the lesional skin of AD patients [14]. More recently, it was shown that patients with Netherton syndrome (NS), a severe ichthyosis in which affected individuals have a significant predisposition to atopic disease, have elevated levels of TSLP in their skin [63]. Mechanical disruption of the epidermis results in TNF- α and IL-1 β production, which are inflammatory cytokines known to induce TSLP expression via NF- κ B [19]. Furthermore, barrier disruption and/or altered barrier function can lead to increased contact with microbes [64]. This will further enhance inflammation and TSLP expression, both directly and indirectly owing to enhanced inflammatory cytokine production. Studies with human skin explants demonstrated

that a combination of inflammatory cytokines, IL-1 β or TNF- α , and the Th2 cytokines, IL-4 or IL-13, is sufficient to induce TSLP expression [65]. These data suggest that TSLP present in the lesional skin of AD patients is the result of skin-resident cell populations responding to inflammatory cytokines, mechanical disruption or microbial products due to defects in barrier function. Recent work in mouse models has demonstrated that TSLP can be produced in the absence of extrinsic stimuli, suggesting cell-autonomous induction of TSLP may also result in the initiation of AD (discussed in the following section).

The mechanisms for TSLP-mediated Th2 skewing of T cells through specific activation of DCs is thought to be the same for both the lung and the skin (as described previously). However, specifically important to AD, TSLP-treated Langerhans cells (TSLP-LCs) freshly isolated from the skin display a slightly different phenotype compared with TSLP-DCs, with upregulation of CD83 and CD86, but not CD80 or CD40 [66]. Both TSLP-LCs and TSLP-DCs are capable of priming naive CD4⁺ T cells towards an inflammatory Th2 phenotype [14,66].

Interestingly, the ability of TSLP-DCs to drive Th2 differentiation is in part due to the lack of IL-12 induction by TSLP [14,60]. In addition to the absence of IL-12, OX40L expressed by the TSLP-DCs is required for induction of the inflammatory Th2 cell phenotype [42]. TSLP has also been shown to induce Th2 cytokine production by mast cells when applied in combination with TNF- α , suggesting that TSLP not only shapes the adaptive immune response, but also influences the innate immune system towards a type 2 response in an inflammatory setting [44]. This phenomenon is supported by mouse models of TSLP overexpression, which will be discussed in the next section.

These findings in human systems suggest a model whereby the epithelial cell dictates the nature of an immune response by producing cytokines, such as TSLP, which act on skin-resident DCs and promote an inflammatory Th2 response. In addition to driving an adaptive Th2 response via programming DCs to promote Th2 cell development, TSLP also acts on tissue-resident mast cells and can potentially mediate inflammation in the absence of adaptive immunity. Mouse models of TSLP-driven AD have elucidated a variety of aspects of human AD, and how TSLP is able to shape both the innate and adaptive responses in the context of AD. It will be important to incorporate known SNPs that predispose humans to AD into mouse models in order to further our understanding of the etiology of AD and to develop targeted therapeutics. Furthermore, it will be important to address the potential of TSLP as a direct therapeutic target in human AD.

TSLP & skin inflammation in mice

A variety of mouse models have demonstrated a role for TSLP in the development of an AD-like disease. For example, conditional overexpression of TSLP by keratinocytes results in an AD-like disease including dry and scaly skin, elevated serum IgE, and Th2 cell and eosinophilic infiltration of the skin [67]. Furthermore, keratinocyte-specific ablation of the nuclear receptors RXR α and RXR β , as well as treatment with vitamin D analogs, results in increased TSLP expression in the skin and a similar AD-like phenotype to that which occurs in mice that are engineered to conditionally overexpress TSLP in a keratinocyte-specific fashion [22,24]. These findings suggest that TSLP expression is repressed by RXRs in the steady state, and derepression occurs in the presence of RXR ligands, such as vitamin D analogs. In addition, these findings support the hypothesis that intrinsic keratinocyte defects can result in TSLP expression in the absence of extrinsic factors, such as inflammatory cytokines or increased microbial contact. It has been unclear whether TSLP is able to initiate AD in humans in the absence of environmental stimuli. However, a recent publication using a mouse model of the human disease NS (*Spink5*^{-/-} mice), showed that TSLP was present in the embryonic skin of mutant mice, a sterile setting that lacks environmental allergens, suggesting that TSLP expression is induced in a cell-intrinsic manner. Furthermore, the investigators found that

Spink5^{-/-} skin grafted onto athymic nude mice experienced significant inflammatory infiltration [63]. These data support a role for TSLP in initiating AD in humans in the absence of extrinsic stimuli. This model also brings to light the ability of TSLP to induce inflammation in the skin in the absence of a functional adaptive immune response, as nude mice lack mature T cells.

Mouse models of AD have elucidated a role for TSLP in driving inflammation in the absence of the adaptive immune response. Chronic elevation of TSLP levels in the skin due to transgenic overexpression or treatment with vitamin D3 analogs results in an AD-like disease in the absence of $\alpha\beta$ T cells [67], or the absence of both T and B cells in RAG-deficient mice [22]. This is most likely due to chronic activation of skin-resident mast cells and the recruitment of other inflammatory cells, such as eosinophils. Interestingly, intradermal injection of recombinant TSLP (rTSLP) over a 2-week period results in local skin inflammation that does require the adaptive immune system, as RAG-deficient mice are resistant to inflammation in this model [50]. This could be due to the reduced bioactivity of rTSLP as compared with endogenous TSLP, or due to the chronic and systemic nature of TSLP production in the transgenic models.

To further clarify the relevant source of TSLP during an allergic response, mice containing a conditional null allele of *tslp* were generated [68]. Crossing this mouse with a keratinocyte-specific Cre-expressing transgenic mouse revealed that production of TSLP by keratinocytes is required for vitamin D3-induced TSLP expression and the development of an AD-like disease [68]. It will be important to determine whether keratinocytes, and epithelial cells in general, are the primary source of TSLP in other models of skin allergy, or if other cell types also serve as sources of TSLP. In a murine model of allergen sensitization with the protease allergen papain, it was demonstrated that basophils accumulate in lymph nodes and produce TSLP [69]. Therefore, it is conceivable that different cell types are capable of producing TSLP in different contexts. This question highlights the finding that both human and murine CD4⁺ T cells are capable of responding directly to TSLP *in vitro*; however, the outcome and context of this response differs from humans to mice [70,71]. Human CD4⁺ T cells express the TSLPR after T-cell receptor stimulation, and costimulation and TSLP treatment results in enhanced proliferation [71]. However, naive murine CD4⁺ T cells are able to respond to TSLP, resulting in IL-4 expression [70]. Naive CD4⁺ T cells primarily localize in lymph nodes, where they may be exposed to TSLP-producing basophils, driving the allergen-specific naive T cells towards a Th2 phenotype. This suggests a distinct mechanism by which TSLP promotes Th2 differentiation in human versus murine systems. In humans, the primary target of TSLP appears to be DCs, which are capable of inducing differentiation of inflammatory Th2 cells [14]. However, in mice it is less clear which cell types are required to respond to TSLP to drive a Th2 response, whether it be DCs, basophils or CD4⁺ T cells. The pertinent responding cell type(s) is most likely context dependent.

A direct action of TSLP on CD4⁺ T cells *in vivo* was recently demonstrated in a delayed-type hypersensitivity (DTH) model [72]. The investigators found that *in vitro* antigen-restimulated CD4⁺ T cells from OVA-sensitized TSLPR^{-/-} mice resulted in reduced levels of the Th2 cytokines IL-4 and IL-13 at the site of OVA challenge. This defect in cytokine production was not due to an inability to proliferate or traffic to OVA-challenged sites, as normal numbers of TSLPR^{-/-} CD4⁺ T cells were present at challenge sites [72]. These data suggest that TSLP acts directly on the CD4⁺ T cells in the skin, promoting Th2 cytokine production, while playing no role in skin homing or proliferation.

Our group also observed a defective Th2 DTH response to hapten allergen in TSLPR^{-/-} mice. This appears to be due to a deficiency in antigen-bearing skin-derived DC accumulation in skin-draining lymph nodes and a reduced ability to drive proliferation of CD4⁺ T cells [Larson

RP, Ziegler SF, Submitted manuscript]. In addition, we observe no requirement for CD4⁺ T cells to respond to TSLP for the development of a Th2 DTH response [Larson RP, Ziegler SF, Submitted manuscript]. Taken together, these data suggest TSLP has pleiotropic effects on adaptive immunity, modulating the response at the level of the DC as well as the T cell.

The 'atopic march' describes the progression of atopic disease in individuals from AD to asthma, as it has been shown that 50% of people with AD go on to develop asthma. Recent and ongoing studies in murine models have demonstrated a role for TSLP in the atopic march. Specifically, mice concurrently subjected to topical vitamin D3 application, or mice lacking RXR α and β , and the OVA/Alum model of asthma experienced exacerbated asthma compared with mice treated topically with vehicle [73]. Similar observations were made in mice with epidermal ablation of recombination signal binding protein for Ig κ J, which develop an AD-like disease owing to elevated TSLP expression [74].

Expert commentary

The role of TSLP in allergic inflammation is currently an area of intense investigation. The data, as reviewed previously, strongly suggest that TSLP plays a critical role in the induction of allergic disease. However, its potential role in disease progression is still somewhat unclear and needs to be elucidated. Equally important, the role that TSLP plays in normal immune homeostasis remains to be determined. This latter point is critical as it may help to determine whether TSLP blockade for the treatment of allergic diseases will be tolerated. Finally, it is now becoming clear that TSLP acts in concert with two other cytokines, IL-17E/IL-25 and IL-33, to drive Th2-type inflammation [30]. Deciphering how these cytokines 'talk' to each other, and how they are individually and collectively regulated, is critical.

Five-year view

The past 5 years has been a very exciting time for research into the role of TSLP in allergic inflammatory responses. The next 5 years will be a critical time for determining whether TSLP is a valid therapeutic target for the treatment of these diseases. Important issues that remain unresolved, but are the focus of ongoing research, include whether TSLP is involved in disease progression, what role TSLP plays in normal immune homeostasis, and the mechanism by which TSLP regulates both Th2- and Th1-type inflammation. It is quite possible that during the next 5 years, therapies for modulating TSLP levels will be approved and used clinically.

Key issues

- Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine whose receptor (TSLP receptor) is a heterodimer of the TSLP binding chain and IL-7R α .
- Unlike other members of the cytokine/cytokine receptor family, TSLP receptor engagement does not lead to tyrosine phosphorylation of the receptor subunits, nor does it lead to Janus protein tyrosine kinase activation. However, it does lead to activation of STAT5.
- TSLP is normally expressed by epithelial cells at barrier surfaces (i.e., skin, lung and gut) and is involved in maintaining homeostasis at these surfaces.
- TSLP expression is elevated at sites of Th2-type allergic inflammation in humans with atopic dermatitis and asthma.

- Mice engineered to lack TSLP responses fail to develop disease in models of allergic inflammation, while mice with tissue-specific overexpression of TSLP develop spontaneous allergic-type disease.
- TSLP can both promote Th2 responses and inhibit Th1 responses.
- TSLP expression is induced by a variety of agents, including inflammatory cytokines, respiratory viruses and bacterial components capable of activating NF- κ B.

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