

REVIEW

Thymic stromal lymphopoietin, OX40–ligand, and interleukin–25 in allergic responses

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Summary

Allergic diseases are often triggered by environmental allergens that induce dominant type 2 immune responses, characterized by the infiltrated T-helper type 2 (TH2) lymphocytes, eosinophils, and elevated TH2 cytokines. In addition to TH2 type immune responses, epithelial stress and injury linked to tissue remodelling are often observed, suggesting that epithelial cells may play important role in regulating allergic responses. Dendritic cells (DCs), the professional antigen-presenting cells with the capabilities of sampling allergens, are considered as the key player on instructing TH2 immune responses. Whether inflamed epithelium can regulate innate immunity, such as macrophages and DCs, which in turn instructs adaptive immunity has long been hypothesized. Studies of thymic stromal lymphopoietin (TSLP), an epithelial cells-derived cytokine, that can strongly activate DCs, provide important evidences that the epithelial barrier can trigger allergic diseases by regulating immune responses. The finding that OX40/OX40Ligand (OX40L) interactions are the molecular trigger responsible for the induction and maintenance of TH2 responses by TSLP-activated DCs provides a plausible molecular explanation for TSLP-mediated allergy. Recent progresses in characterizing the pro-inflammatory IL-17 cytokine family have added an additional layer of complexity on the regulation of allergic inflammation. TSLP–DCs can induce a robust expansion of TH2 memory cells and strengthen functional attributes by up-regulating their surface expression of IL-17RB (IL-25R), the receptor for cytokine IL-17E (IL-25), a distinct member of IL-17 cytokine family. IL-17E (also known as IL-25) produced by epithelial cells, and other innate cells, such as eosinophils, basophils, and mast cells, are shown to regulate adaptive immunity by enhancing TH2 cytokine productions. These exciting findings expand our knowledge of the complex immunological cascades that result in allergic inflammation and may provide novel therapeutic approaches for the treatment of allergic diseases.

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Introduction

Allergic responses induced by dysregulated type-2 immune response to environmental allergens often cause harmful symptoms such as asthma and atopy. The epithelial barrier in the local mucosal surface such as airway, skin, and gastrointestinal has long been hypothesized to play important roles in the initiation of allergic response by secreting various chemokines, cytokines, and growth

factors, which in turns regulate innate immune cells. The idea that the epithelial barrier and altered innate immunity are fundamental to the onset of allergic diseases is supported by the findings that thymic stromal lymphopoietin (TSLP) represent a key molecule at the epithelial cell–dendritic cell (DC) interface to initiate allergic inflammation. Upon exposure to allergens or virus infections, pro-inflammatory cytokines, such as TNF- α and IL-1 β , trigger strong TSLP production by human airway epithelial cells and human keratinocytes. In turn, TSLP endows DCs to induce the differentiation of inflammatory T-helper type 2 (TH2) cells and the expansion and activation of allergen-specific TH2 memory cells. In addition,

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understanding the maintenance and the regulation of long-lived allergen-specific human TH2 memory cells and their molecular signatures may provide novel therapeutic approaches for the treatment of allergic diseases. Recent advances demonstrate that cytokines TSLP and IL-25, as well as OX40Ligand (OX40L)/OX40, a member of TNF/TNF receptor superfamily, seem to be important contributors in the maintenance of TH2 memory pool during the pathogenesis of allergic inflammation. In this review, we discuss how these three factors contribute to the onset and maintenance of allergic response by regulating innate and adaptive immunity.

Thymic stromal lymphopoietin and thymic stromal lymphopoietin receptor

TSLP, a distant paralog of IL-7, is a type I cytokine that is part of the IL-2 'cytokine family' (IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-21, and TSLP) [1]. Murine TSLP was first identified as a factor expressed in the supernatants of thymic stromal cell line that could support the development of B cells [2]. The activities of mouse TSLP overlap with those of IL-7, which can stimulate the proliferation of thymocytes and facilitate B lymphopoiesis in cultures of fetal liver and bone marrow lymphocyte precursors. Subsequent characterization and cloning showed that the activity was a result of four-helix bundle cytokine with three potential sites for N-linked carbohydrate addition and seven cysteine residues [1]. Human orthologue of TSLP was later discovered in a computational screen of genomic database. While human TSLP shares poor homology with that of mouse at only 43% amino acid identity, sequence prediction of human TSLP cDNA showed a similar four-helix structured cytokine with two *N*-glycosylation sites and six cysteine residues. Both mouse and human TSLP are expressed predominantly by epithelial cells, especially in the lung, skin, and gut, and exert similar biological functions as discussed below.

TSLPR is an atypical type I cytokine receptor and exhibits a remarkable 24% identity to the common γ receptor chain (γ_c) [3]. Similar to other type I cytokine receptor, TSLPR contains the conserved box 1 region, which is important for the binding of Janus family tyrosine kinase. TSLP was found to signal through a heterodimeric receptor complex that consists of a new member of the haemopoietin family termed TSLPR and IL-7R α chain [4, 5]. TSLP binds TSLPR with low affinity, but the addition of IL-7R α chain greatly enhances the binding affinity [3, 4]. While human and mouse TSLPR share only 39% amino acid identity, expression of TSLPR transcript can be detected in early B and T cell progenitors, peripheral CD4⁺ T cells, mast cells, DCs, and myeloid cells in both species [6]. Therefore, TSLP may exert various biological functions by acting on cell lineages in different immunological context [7]. In humans, myeloid DCs were

found to express the highest level of TSLPR in both transcript and protein levels among the examined haematopoietic cell lineages, suggesting that myeloid DCs are the primary responding cells to this epithelial-derived cytokine. Molecular studies in T cells or transfected cell line showed that signalling through the TSLPR/IL-7R α heterodimeric complex results in the activation of signal transducer and activator of transcription (STAT)3 and STAT5 [3, 5, 8]. However, the comprehensive analyses of signalling pathways activated by TSLP within primary myeloid DCs have not been reported (Fig. 1).

Regulation of thymic stromal lymphopoietin expression during allergic inflammation

During allergic inflammation, epithelial cells, keratinocytes, and stromal cells are the primary producers of TSLP [9]. Recent findings show that pro-inflammatory cytokines TNF- α and IL-1 β can synergize with TH2 cytokines (IL-4 and IL-13) to induce TSLP production by human epithelial cells and keratinocytes [10]. Moreover, TLR3 ligand such as a viral double-strand intermediate

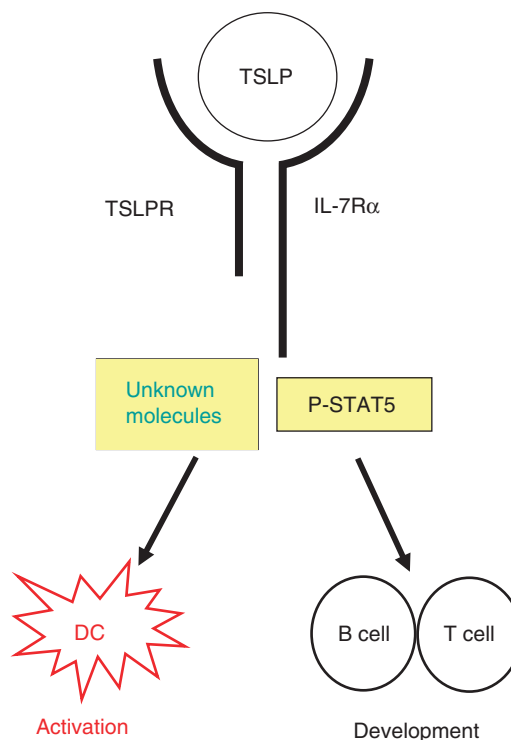


Fig. 1. Thymic stromal lymphopoietin (TSLP) and thymic stromal lymphopoietin receptor (TSLPR) structure and function. TSLP was discovered by its biological activity to promote B and T cell development. In the periphery, TSLP strongly activates myeloid dendritic cells (DCs) that up-regulate their surface TSLPR expression rapidly. The TSLPR is a heterodimeric complex composed of TSLPR and IL-7R α chains. In T cell lines, TSLP stimulation induced strong activation and phosphorylation of STAT5, whereas unidentified molecules or pathways may exist downstream of TSLPR signalling in DCs.

RNA produced during rhinovirus replication can augment TSLP production by TH2 cytokines-primed epithelial cells [11]. The ligands for TLR2, TLR8, and TLR9 can trigger the production of TSLP by human primary epithelial cell lines [12]. In a murine allergic rhinitis model, TSLP production by nasal epithelial cells can be induced by mast cells expressing FcεRI. In addition to structural cells, basophils also have the potential to produce TSLP for the initiation of type-2 immune response in the papain-induced mouse allergic model. These findings suggest that epithelial-derived TSLP expression may be regulated by the inflammatory stimuli produced by innate or adaptive immune cells or some TLR ligands during allergic inflammation.

The molecular mechanism to control TSLP gene expression induced by these inflammatory stimuli or TLR ligands has recently been addressed. One study suggested that the activation of nuclear factor-κB signalling pathway plays a key role on the induction of TSLP gene expression by the inflammatory stimuli [12]. The others demonstrated that retinoid X receptor (RXR)α or RXRβ can pair with vitamin D receptor and act as a transcriptional repressor to inhibit TSLP gene expression in the mouse skin keratinocytes [13, 14]. These studies may have the potential to provide molecular links between allergen exposure and TSLP induction during allergic immune response.

Dendritic cells bridge innate and adaptive responses through effects mediated by thymic stromal lymphopoietin

Because the identification of TSLP cytokine in mouse, many studies have been focused on the effect of TSLP on promoting B and T cell development [6, 15, 16]. The findings that human TSLP can strongly activate peripheral blood CD11c⁺ immature DCs in culture provide a novel biological function of TSLP, linking this epithelial-derived cytokine to DC-mediated allergic immune responses. DCs are the professional antigen-presenting cells (APCs) that have the capability to sense invaded pathogens and tissue stress, then present antigen to T cells, thereby bridging the innate and adaptive immunity. Human DCs express high level of TSLPR and respond to TSLP rapidly by up-regulating their surface expression of MHC class II, CD40, CD80, CD86, and the DC-associated activation marker, DC-lamp [17]. Unlike other DC stimuli such as CD40L and TLR ligands, TSLP does not stimulate mDCs to produce the TH1-polarizing cytokine IL-12 or the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. To identify the unique molecular features of mDCs induced by TSLP but not other stimuli, microarray analysis of mDCs activated with different stimuli were performed [18]. TSLP does not induce mDCs to express mRNA encoded IL-12 family members, such as IL-12, IL-23, and IL-27, nor the type I IFNs, the cytokines known to induce TH1 differentiation. Interestingly, TSLP triggers mDCs to produce an array of distinct chemokines, including eotaxin-2, IL-8, as well as

TARC and I-309, which are important for recruiting eosinophils, neutrophils, and TH2 cells, respectively [19]. These findings suggest that one of the most important features of TSLP cytokine is to endow mDCs to become capable of inducing type-2 immune response, while losing the capability of producing TH1 polarizing cytokines. At the site of allergic inflammation; such as the airway, intestine, and skin, DCs are in close contact with epithelial cells. TSLP may thus mediate the crosstalk between epithelial cells and DCs during allergic inflammation, thus leading to allergic diseases [6]. Indeed, strong TSLP expression by keratinocytes in the atypical layer of the epidermis was found in the lesional skins of patients with chronic atopic dermatitis, providing the first evidence for the association of TSLP expression and allergic diseases. A recent study further demonstrates that the severity of asthma is correlated with the elevation of TSLP expression in the airway of asthmatic subjects [20]. Moreover, studies *in vivo* using genetic approaches in mice showed that tissue-specific over expression of TSLP in lung and skin triggers allergic response leading to asthma and atopic dermatitis, respectively, whereas mice with gene disruption of TSLPR fail to develop airway inflammation in the allergen-challenged animal model [21]. Together, these studies suggest that TSLP produced by epithelial cells can activate DCs to create a TH2 permissive microenvironment leading to asthma and atopy (Fig. 2).

Thymic stromal lymphopoietin-activated dendritic cells induce inflammatory T-helper type 2 cells and maintain T-helper type 2 memory pool

Studies in mice suggest that TSLP may exert direct effects on CD4⁺ T cells. During T cell development in thymus, TSLP may modestly affect thymocyte growth *in vitro* and support an overall population expansion of peripheral lymphocyte *in vivo* by injection of TSLP [16]. While TSLPR-deficient (*Tpte2*^{-/-}) mice have normal T and B cell development [7], *Tpte2*^{-/-}*IL2rg*^{-/-} mice have more deleterious phenotype than that in *IL2rg*^{-/-} mice, suggesting a non-redundant influence of TSLP on T lymphocyte development [16]. Indeed, CD4⁺ T cells from TSLPR-deficient mice expanded less efficiently than wild-type CD4⁺ T cells in irradiated hosts, indicating that TSLP may play a direct role on T cell homeostasis [16]. In humans, pre-activated but not naïve CD4⁺ T cells up-regulate their TSLPR expression and become responsive to TSLP stimulation [22]. Following TCR engagement, TSLP augments the proliferative response of the pre-activated CD4⁺ T cells by activating STAT5 and inducing STAT5 target genes *in vitro* [22]. In addition, studies in mice suggest that the epithelial or basophil-derived TSLP can directly induce TH2 differentiation of naïve CD4⁺ T cells in the presence of anti-CD3 stimulation *in vitro* [23]. While these studies propose that TSLP produced by innate immunity

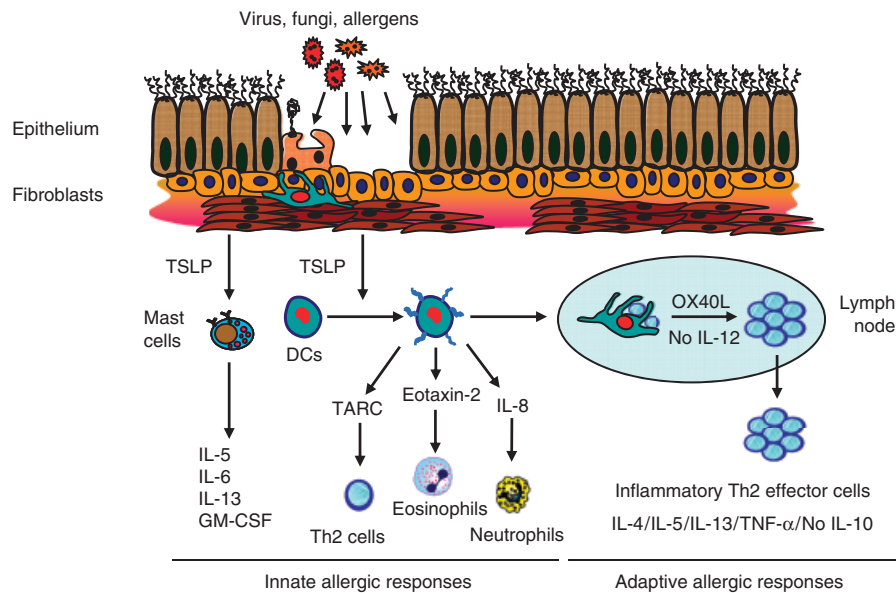


Fig. 2. Thymic stromal lymphopoietin (TSLP) initiate innate and adaptive allergic responses. Invaded pathogens or allergens can trigger mucosal epithelial cells or skin cells (keratinocytes, fibroblasts, and mast cells) to produce TSLP. TSLP can initiate innate allergic responses by activating immature dendritic cells (DCs) to produce chemokines IL-8, eotaxin-2, and T-helper type 2 (TH2) attracting chemokine TARC. TSLP can also costimulate mast cells to produce IL5, IL13, GM-CSF, and IL-6. TSLP-activated mDCs can migrate into the draining lymph nodes to initiate adaptive allergic responses. TSLP-activated DCs express OX40 ligand (OX40L), which triggers the differentiation of naïve $CD4^+$ T cells into inflammatory TH2 cells and the expansion of allergen-specific TH2 memory cells.

can trigger allergic response by directly targeting activated T cells in the absence of DCs, other studies in human and mice suggest that DCs are the primary cells responsible for TSLP-induced allergy.

In humans, TSLP-activated mDCs rapidly up-regulate their surface TSLPR expression at a much higher level compared with that on pre-activated $CD4^+$ T cells [24]. Mature DCs induced by TSLP mediate the differentiation of naïve $CD4^+$ T cells into a unique type of TH2 cells that produce classic TH2 cytokines IL-4, IL-5, and IL-13, and large amount of TNF- α , but little IL-10 (Fig. 2). TSLP-activated DCs can also trigger $CD8^+$ T cells to differentiate into pro-allergic cytotoxic T cells that produce IL-13 [25]. Similar studies in mice also demonstrated that TSLP functions on the maturation and activation of DCs, which in turn, induce the differentiation of naïve $CD4^+$ T cells to TH2 cells were also observed [21, 26]. In addition, TSLP-activated DCs can induce a robust expansion of human TH2 memory cells while maintaining their central memory phenotype and TH2 commitments [27]. TH2 memory cells expanded by TSLP-activated DCs undergo further TH2 polarization and express pro-allergic genes such as IL-25R (IL17RB), cystatin A, Charcot-Leydon crystal protein, and prostaglandin D_2 synthase (Fig. 3) [27]. While some studies in patients with atopic dermatitis observed elevated TSLP expression in inflamed skin [17, 27], others demonstrated that these patients often exhibit significantly increased frequency of circulated $CRTH2^+CD4^+$ TH2 memory cells in blood [28]. Recently, we further showed that patients with AD, but not TH1-mediated

inflammatory skin diseases, exhibited strong TSLP expression together with accumulated $CRTH2^+CD4^+$ TH2 memory cells clustering with DC-LAMP $^+$ DCs within dermis of their lesional skin [27]. Together, these studies led us to propose that at the sites of allergen exposures, DCs activated by epithelial-derived TSLP or other stimuli may function not only in priming TH2-mediated immune responses but also in sustaining the allergic inflammation by inducing the expansion and further TH2 polarization of allergen-specific TH2 memory T cells, thereby contributing to the maintenance and relapse of TH2-mediated allergic diseases [27, 29–31]. The potential for TSLP to provide a new therapeutic target for the treatment of allergic disorders is compelling, and elucidating the mechanisms that regulate TSLP expression and the effects of TSLP on orchestrating the immune response towards a TH2 phenotype should facilitate this quest.

OX40/OX40Ligand: the molecular trigger for thymic stromal lymphopoietin-dendritic cells-induced allergic responses

In an attempt to identify the molecular mechanisms by which TSLP-activated DCs induce allergic inflammation, OX40L was identified to be the key molecule expressed by TSLP-activated DCs using microarray analyses. OX40L, originally termed glycoprotein 34 kDa (GP34), and its cognate receptor OX40, belong to TNF and TNF receptor superfamily [32, 33]. OX40 is preferentially expressed on activated $CD4$ T cells, whereas OX40L is mainly expressed

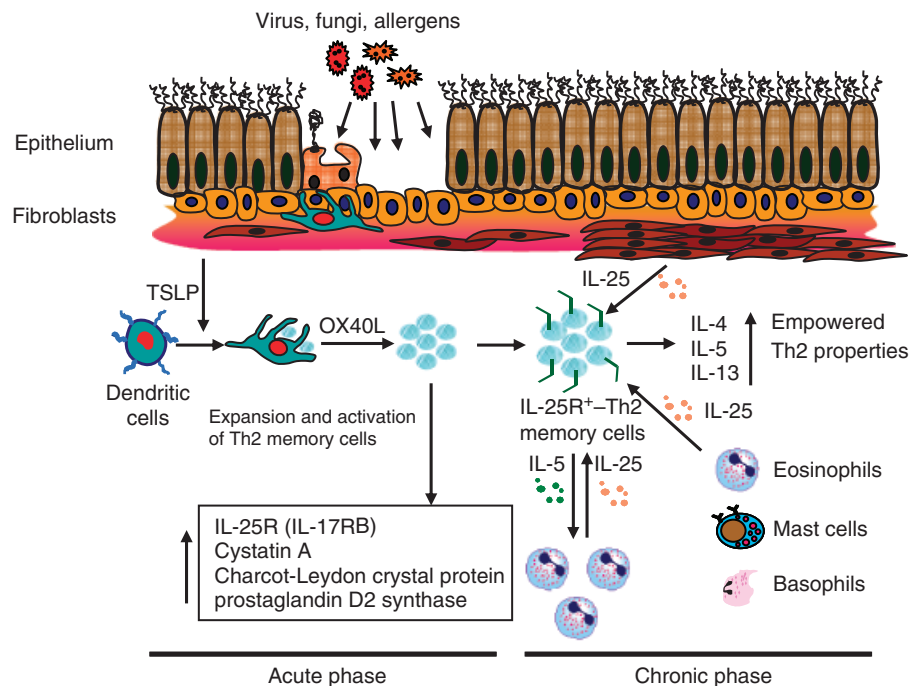


Fig. 3. OX40 ligand (OX40L) and IL-25 (IL-17E) maintain chronic allergic responses. Dendritic cells (DCs) activated by thymic stromal lymphopoietin (TSLP) can expand and activate resident T-helper type 2 (TH2) memory cells that up-regulates the expression of pro-allergic molecules, including IL-25R, cystatin A, Charcot-Leydon crystal protein, and prostaglandin D_2 synthase. During chronic allergic inflammation, infiltrated mast cells, eosinophils, basophils, and injured structural cells can produce IL-25 that can further enhance TH2 cytokines production, in particular IL-5 and IL-13 by DC-activated TH2 memory cells.

by APC, including activated DCs, B cells, macrophages, and Langerhan cells, as well as T cells and endothelial cells. Unlike other costimulatory molecule; such as CD28 that plays an important role on T cell priming, OX40 is not constitutively expressed on naïve T cells but is induced at 24–48 h after engagement of TCR, with peak levels between day 2 and day 5 *in vivo* after antigen immunization. OX40-deficient $CD4^+$ T cells proliferate initially, but cannot survive long-term due to lower expression of anti-apoptotic proteins such as Bcl-2, Bcl-xL, and Bfl-1. Therefore, OX40/OX40L interaction was shown to be crucial for T cell activation, and survival and the generation of memory T cells from activated effector T cells [34].

The interaction between OX40 and OX40L is implicated in a number of inflammatory diseases, in particular allergic inflammation. In the studies of allergen-induced animal models, mice lacking OX40 or OX40L exhibit significant impairments of TH2 responses and diminished lung inflammation, implicating the involvement of OX40 signalling on triggering allergic response [35, 36]. The finding that OX40L expression on TSLP-activated DCs plays an important role for the induction of inflammatory TH2 cells provides the molecular basis for the understanding of OX40/OX40L interaction on the induction of allergic responses. Blockade of OX40 and OX40L interactions using a neutralizing antibody against OX40L inhibited the production of TH2 cytokines and TNF- α and

enhanced the production of IL-10 by the differentiating $CD4^+$ T cells cocultured with TSLP-activated DCs. OX40L-induced inflammatory TH2 cell differentiation depends on the absence of IL-12, as OX40L is incapable of triggering inflammatory TH2 cell differentiation in the presence of IL-12. Thus TSLP-activated DCs may create a TH2-permissive microenvironment by up-regulating OX40L without inducing the production of TH1-polarizing cytokines. Moreover, OX40L expressed by TSLP-DCs also plays an important role on driving the expansion of TH2 memory cells by contributing to the prolonged cognate formation during T cell–DC interaction [27]. Loss of OX40 signalling during T cell–DC interaction induces the expression of p14^{ARF} and other CDK inhibitors in $CD4^+$ TH2 memory cells, which results in cell cycle arrest or cell senescence [27]. Triggering OX40 signalling in TH2 memory cells by their ligands on TSLP–DCs is indispensable for the reactivation and homeostatic maintenance of the TH2 memory pool by controlling their entrance into cell cycle, thereby linking the critical role of OX40L in the pathogenesis of allergic inflammation in animal models [36]. Together, these studies provide the plausible explanations for the functions of OX40L on inducing the generations of inflammatory TH2 cells and the maintenance of TH2 memory pool during TSLP–DCs-mediated allergic response [37].

The pivotal role of OX40L on TSLP-induced allergic inflammation is further demonstrated in a recent study

using both mouse and non-human primate models *in vivo*. To address the role of OX40/OX40L interactions in contributing to allergic response in both animal models, the chimeric hamster-mouse mAb and a fully humanized mAb against mouse, and human OX40L, respectively, were generated [38]. The efficacy of these mAb treatments was demonstrated by their ability in abolishing the interactions of OX40/OX40L and the depletion of OX40L-positive DCs [38]. These invaluable anti-OX40L antibodies prove to be efficacious in inhibiting antigen driven TH2 inflammation in mouse and non-human primate model of asthma. Administrations of these anti-OX40L antibodies resulted in a significant reduction of TH2 cytokines, and antigen-specific IgE and IgG1, and the loss of infiltrated eosinophils and CD4⁺ effector/memory T cells [38]. Interestingly, the treatments resulted in only moderate reduction of TH2 inflammatory response during a primary effector response, whereas a significant decrease of re-activation and infiltration of memory CD4⁺ T cells, TH2 cytokine production, and antigen-specific serum IgE levels were observed during antigen recall response. These results demonstrated the critical roles of OX40/OX40L interaction on the maintenance and re-activation of TH2 memory responses during TSLP-DCs-mediated allergic responses (Fig. 3). Targeting the TSLP-DC-induced inflammatory TH2 cells or allergen-specific TH2 memory cells by blocking OX40/OX40L interaction may be one of the new therapeutic approaches for the prevention and treatment of human-allergic diseases.

Strong induction of interleukin-17RB, a cognate receptor for interleukin-25 (interleukin-17E), in T-helper type 2 memory cells by thymic stromal lymphopoietin-dendritic cells

Studies from patients and animal experimental models indicate that memory-like TH2 cells that reside in the lungs during disease remission can become activated effectors upon allergen exposure and are one of the principle cells responsible for the exacerbation of allergic diseases. One of the unique features of TSLP-induced allergic responses is the maintenance and activation of TH2 memory cells. TSLP-DCs induce strong up-regulation of IL-17RB (IL-25R) expression by activated TH2 memory cells, but not in other T cell subsets [39], thereby linking TSLP-driven TH2 responses to the functions of IL-17E (IL-25), a distinct member of IL-17 cytokine family. IL-17RB serves as co-receptor for both IL-17B and IL-17E (IL-25), with higher binding avidity to IL-17E (also known as IL-25) [40]. IL-25 belongs to a recent discovered IL-17 cytokine family, which consist of IL-17A, and five additional family members designated as IL-17B-F [41–43]. IL-17A identified from activated T cell clones (originally named as CTLA-8) is the prototypic family member [44–46]. Among the IL-17 cytokine family, the expression

and functions of IL-17A, IL-17F, and IL-17E (IL-25) are better characterized. IL-17F shares the greatest similarity with IL-17A (55% identity), whereas IL-17E (IL-25) is the most distant (17%) [47]. Studies of IL-17 receptor family (IL-17RA-E) showed additional complexities in the regulations and biological functions of IL-17 cytokine family. IL-17RA, the cognate receptor for IL-17A, is ubiquitously expressed [48]. However, the biological activity of IL-17A or IL-17F is dependent on the heterodimeric complex composed of IL-17RA and IL17RC [49]. In asthmatic patients, IL-17A expression was increased in the lungs, sputum, bronchoalveolar lavage fluids or sera, and the severity of airway hypersensitivity in patients correlates with the level of IL-17A expression, suggesting that IL-17 cytokines play important roles in driving allergic inflammation [50]. Indeed, IL-17A and/or IL-17F can orchestrate local inflammation by inducing the release of pro-inflammatory cytokines such as TNF- α , IL-1 β , G-CSF, and IL-6, as well as chemokines CXCL1/Gro- α , CXCL2, and CXCL8/IL-8 production by human bronchial fibroblast, epithelial, and airway smooth muscle cells, as well as venous endothelial cells *in vitro* [51–53]. Studies in mice showed that IL-17R-deficient mice exhibit not only reduced neutrophil but also eosinophil recruitments [54], whereas IL-17A^{-/-} mice exhibited reduced TH2 responses to antigen sensitization [55]. As summarized in Table 1, these studies suggest that IL-17A/F can trigger lung inflammation by stimulating innate immunity to mediate neutrophil recruitments, implicating the potential role of IL-17A/F on the pathogenesis of severe asthma mediated by neutrophilia.

Distinct from other IL-17 cytokine family members, IL-25 (IL-17E) was first described as a TH2 cell-derived cytokine [56]. Human IL-25 shares 80% identity with mouse transcript and exhibits as two isoforms [56]. The isoform 1 has an extended N-terminal sequence and is expressed at high level in the testis, trachea, and brain, whereas isoform 2 is mainly expressed in the testis, prostate, and spleen. In mice, IL-25 transcript can be

Table 1. IL-17 cytokine family and functional effects on allergy

Ligands	Receptors	Functions	References
IL-17A	IL-17RA	IL-6, IL-8, IL-11, Gro- α , G-CSF, and GM-CSF $\uparrow$	[53]
IL-17F	IL-17RA/C	MUC5AC and MUC5B $\uparrow$	[52]
		Airway hyper-reactivity $\uparrow$	[55]
		Neutrophilia $\uparrow$	[51]
		Severity of asthma $\uparrow$	[55]
IL-17E	IL-17RB	IL-4, IL-5, IL-13, IgE, and eotaxin $\uparrow$	[39, 56, 58–60, 64]
		Mucus secretion $\uparrow$	[60, 61]
		Airway hyper-reactivity $\uparrow$	[56, 62, 64]
		Eosinophilia $\uparrow$	[56, 58, 60, 64]
		Severity of asthma $\uparrow$	[56, 60, 62, 64]

found in the gastrointestinal tract and uterus, and IL-25R is expressed in the lymphoid organs, lung, and liver [57]. Recent studies demonstrated that the expression of IL-25 transcript could be found in the mast cells activated by IgE cross-linking [58], alveolar macrophage and lung epithelial cells stimulated with allergens in mice [59]. In humans, bioactive IL-25 protein was found to be secreted by activated eosinophils and basophils [39]. Interestingly, these cells obtained from allergic patients produce more substantial amounts of IL-25 after activation. These studies suggest that multiple cellular sources of IL-25 may exist during allergic responses (Fig. 3).

Interleukin-25 enhances allergic response through multiple pathways

Compared with other IL-17 cytokine family members, IL-25 is distinctive with low sequence homology, unique expression pattern, and unique functions in evoking type-2 immune responses in animal studies [56, 60–62]. Systemic administration of IL-25 protein [56, 62] or over expression of IL-25 [60, 61] induces elevated TH2 cytokine and eotaxin productions, which result in eosinophilia, increased serum IgE, mucus hyperplasia, and other pathological changes in many tissues. Moreover, administrations of neutralizing antibody against IL-25 in an experimental model of allergic asthma resulted in significantly reduced levels of IL-5, IL-13, and serum IgE production, the infiltration of TH2 cells and eosinophils, and prevented airway hyperresponsiveness [63]. These *in vivo* studies imply that IL-25 may play a pivotal role in the development of TH2-mediated allergic inflammation.

The function of IL-25 on type-2 immunity, which play protective role in defense against parasitic infection was further elucidated by recent studies in animal models using helminth infection. In the absence of IL-25, mice infected with *Trichuris muris*, the gastrointestinal parasite, failed to develop a lymphocyte-dependent protective type 2 immunity to expel chronic parasitic infection [64]. In other study, IL-25 was found to trigger the non-B or -T, c-kit⁺ cells for the rapid clearance of *Nippostrongylus brasiliensis* acute infection [65]. Using allergen-induced allergic animal models, one study showed that the administrations of recombinant IL-25 proteins can induce acute lung inflammation mediated by the unidentified IL-5-producing non-B or -T cells [62], whereas the other demonstrated that the enforced expression of IL-25 in lung resulted in the amplification of allergic inflammation driven by CD4⁺T cells and STAT6 signalling pathway [66]. These findings suggest that IL-25 can enhance type-2 immune responses by regulating either CD4⁺ T cells or non-B or -T, c-kit⁺ cells depending on experimental models.

The finding that IL-25 receptor (IL-25R or IL-17BR) is highly expressed on CD4⁺ TH2 memory cells in humans has provided direct evidence that IL-25 can function

directly on CD4⁺ T cells to mediate enhanced type-2 immune response [39]. Indeed, IL-25 co-stimulates the proliferation of the TH2 memory cells, and enhances their TH2 polarization and cytokine productions, in particular IL-5, by up-regulating the gene expression of the transcription factors, *GATA-3*, *c-MAF*, and *junB* in an IL-4 independent manner [39]. A parallel study in mouse showed that IL-25 treatment during T cell differentiation can enhance TH2 cytokine productions and inhibit IFN- γ production, indicative of the TH2 polarizing function [59]. Together, these results suggest that IL-25 may amplify allergic immune responses by inducing the local expansion and augmented effector functions of TH2 memory/effector cells. On the contrary to the T cell derived pro-inflammatory cytokine IL-17A and IL-17F, which induce the productions of pro-inflammatory cytokines/chemokines from a variety of cell types during the onset of allergic inflammation, IL-25 (IL-17E) produced by activated eosinophils, basophils, and mast cells may exert a critical role in maintaining the functional capacity and maintenance of IL-25R-expressing allergen-specific TH2 memory cells, thus propagating a positive feed back loop between innate effectors and adaptive immunity leading to the amplification of allergic inflammation (Fig. 3).

Conclusions

TSLP is a cytokine with pleiotropic functions. TSLP appears to function in CD4⁺ T cell homeostasis in the peripheral mucosa-associated lymphoid tissues under normal physiological conditions, whereas aberrant TSLP expression by epithelial cells or keratinocytes has been shown to play a critical role in inducing allergic inflammatory diseases, such as asthma and atopic dermatitis. Invaded pathogens, allergens, or other environmental factors that can induce pro-inflammatory cytokine productions have the potential to trigger the strong TSLP expression in the epithelium layer. The RXR α or RXR β that can pair with vitamin D receptor to act as a transcriptional repressor to control TSLP gene expression may be one of the crucial factors to control the steady state level of TSLP production. TSLP targets mDCs to create a TH2 permissive microenvironment at local inflammatory sites. TSLP-activated DCs are potent inducers in the generation of inflammatory TH2 cells and the maintenance and activation of TH2 memory cells via their surface OX40L expression. TSLP-activated DCs induce activated TH2 memory/effector cells to express the high level of surface IL-25R, thereby responding to the stimulation of IL-25 to produce elevated TH2 cytokines. The eosinophil/basophil lineage, particularly eosinophils, appears to be the potential producers of the cytokine IL-25. This raises the possibility that eosinophils, through the production of IL-25, may exert a critical role in maintaining the functional capacity of allergen-specific T central memory cells

in allergic inflammation. These recent exciting findings have not only advanced our understandings of complex allergic responses but also provided important pointers towards novel approaches in the prevention and therapy for allergic diseases.

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