

The Role of Thymic Stromal Lymphopoietin in Allergic Inflammation and Chronic Obstructive Pulmonary Disease

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Abstract Thymic stromal lymphopoietin (TSLP) primes dendritic cells to promote a Th2 inflammatory response. Its action is mediated by a heterodimeric receptor which consists of the interleukin-7 receptor α chain and the TSLP receptor chain (TSLPR). TSLPR resembles the common γ chain subunit utilized by many type 1 cytokine receptors. Normal epithelial cells, keratinocytes, and stromal cells constitutively express TSLP. Dendritic cells that are activated by TSLP promote the development of CD4⁺ T cells into pro-inflammatory Th2 cells. TSLP thus plays a potentially important role in the pathogenesis of allergic inflammation in asthma and atopic dermatitis. TSLP also has direct effects on other types of cells in the bronchial mucosa. It is over-expressed in the bronchial mucosa in chronic obstructive pulmonary disease (COPD), which is traditionally described as a Th1-related disease, as well as severe asthma, which is traditionally described as a Th2-related disease. In this review we will discuss TSLP expression, function, and available and potential mechanisms in both allergic inflammation and COPD.

Keywords TSLP · Asthma · COPD

Abbreviations

AD	Atopic dermatitis
BAL	Bronchoalveolar lavage
CCL	Chemokine (C–C motif) ligand
COPD	Chronic obstructive pulmonary disease
DC	Dendritic cell
DEP	Diesel exhaust particle
dsRNA	Double-stranded ribonucleic acid
HASMC	Human airway smooth muscle cell
HBEC	Human bronchial epithelial cell
IL	Interleukin
IL-7R α	IL-7 receptor α chain
JAK	Janus family tyrosine kinases
LC	Langerhans cell
LPA	Lysophosphatidic acid
LTA	Lipoteichoic acid
MDC	Macrophage-derived chemokine
NF- κ B	Nuclear factor- κ B
NK	Natural killer
OX40L	OX40 ligand
poly I:C	Polyinosinic:polycytidylic acid
RSV	Respiratory syncytial virus
STAT	Signal transducer and activator of transcription
TARC	Thymus activation-regulated chemokine
Th	T-helper
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	T-regulatory
TSLP	Thymic stromal lymphopoietin
TSLPR	TSLP receptor chain
TSLP-DC	TSLP-activated dendritic cell
TSLP-LC	TSLP-activated Langerhans cell
γ c	Common γ chain
VDR	Vitamin D receptor

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Introduction

Asthma and atopic dermatitis (AD) are allergic inflammatory disorders characterized by the infiltration and accumulation of memory-like T-helper (Th)2 cells and eosinophils (Kay 2001). The inflammatory profile in these disorders is predominantly allergen-driven Th2-coordinated inflammation with the release of interleukin (IL)-4, IL-5, IL-9, and IL-13. Up-regulation of the cytokines IL-4 (Leung et al. 2005; Matsunaga et al. 2006; Quaedvlieg et al. 2006; Shahid et al. 2002), IL-5 (Kim et al. 2003), IL-9 (Shimbara et al. 2000), and IL-13 (Bodey et al. 1999; Huang et al. 1995) can be detected in exhaled breath condensate, sputum, bronchoalveolar lavage (BAL) fluid, and endobronchial biopsies of asthmatics. These cytokines play a key role in allergic inflammation and remodeling in asthma, such as inducing IgE production and vascular cell adhesion molecule-1 expression, maturation of eosinophils, mast cell development, mucus overproduction, and airway hyperresponsiveness (Corry 2002; Kay 2003). The Th2 cytokine release pattern is also of pathophysiological importance in AD, at least in the initial stages of AD (Incorvaia et al. 2008; Numerof and Asadullah 2006).

As antigen-presenting cells, dendritic cells (DCs) play a critical role in directing T cell responses to an antigen, priming T cells to develop into Th1, Th2, and Th17 memory/effector cells. DCs activated by thymic stromal lymphopoietin (TSLP) *in vitro* promote the development of Th2 memory/effector cells (Liu et al. 2007). The involvement of TSLP in the Th2 inflammatory pathway is suggested by the fact that the expression of TSLP is increased in the bronchial mucosa of asthmatics and its expression is correlated with Th2-attracting chemokine expression and disease severity (Kato and Schleimer 2007; Ying et al. 2005). The role of TSLP in chronic obstructive pulmonary disease (COPD) is less clear, although its expression at the mRNA and protein level is elevated in COPD (Ying et al. 2008). This suggests that TSLP not only plays a key role in allergic inflammation, but may also play a role in non-allergic airway inflammation. In this review we will discuss the factors and mechanisms involved in the expression of TSLP, the function and molecular mechanisms underlying TSLP's role in allergic inflammation, and the potential role and mechanism of TSLP in COPD.

TSLP/TSLP Signaling Pathway

TSLP, an IL-7-like cytokine, was first isolated from the supernatant of a murine thymic stromal cell line and initially recognized as a pre-B-cell growth factor (Friend et al. 1994; Ray et al. 1996). Despite exhibiting poor homology (amino-acid correspondence is 43%), human and murine

TSLP exert similar biological functions (Liu et al. 2007; Quentmeier et al. 2001). In humans, TSLP is expressed predominantly by epithelial cells (mostly of the lung, skin, and gut) and keratinocytes (Allakhverdi et al. 2007; Liu et al. 2007). Lung fibroblasts, DCs, mast cells, and smooth muscle cells all have the potential to produce TSLP (Liu et al. 2007; Soumelis et al. 2002). TSLP is also produced by allergen-activated basophils (Sokol et al. 2008), but not by most other hematopoietic cells, including B cells, T cells, natural killer cells, granulocytes, and macrophages (Liu et al. 2007; Zhang et al. 2007).

The TSLP receptor is a heterodimeric receptor consisting of the IL-7 receptor α chain (IL-7R α) and a specific subunit, the TSLP receptor chain (TSLPR). The IL-7R, on the other hand, is a heterodimer of IL-7R α and the common γ chain (γ_c). Human and murine TSLPR share 39% amino-acid identity, while human TSLPR and human γ_c share 24%. Co-expression of human TSLPR and IL-7R α is found in activated mast cells, CD4⁺ T cells, CD8⁺ T cells, DCs, and monocytes (Allakhverdi et al. 2007; Park et al. 2000; Reche et al. 2001). The TSLPR, like its murine counterpart (see below), has been found to induce phosphorylation of signal transducer and activator of transcription 5 (STAT5) in the absence of Janus family tyrosine kinase 3 (JAK3) activation (Quentmeier et al. 2001; Reche et al. 2001). Human TSLP receptor is also able to utilize STAT3 (Reche et al. 2001).

In contrast to humans, murine TSLPR (mTSLPR) is expressed in many tissues, including liver, brain, heart, kidney, testis, bone marrow, spleen, and thymus, and on a variety of cell types, including T cells, B cells, DCs, and monocytes (Pandey et al. 2000; Park et al. 2000; Tonozuka et al. 2001). mTSLPR is similar to the γ_c subunit, with a 47% similarity at the protein level (Pandey et al. 2000; Park et al. 2000; Tonozuka et al. 2001). TEC family kinases might be involved in TSLP-mediated STAT5 activation, while JAKs might be involved in mIL-7 signaling (Isaksen et al. 1999). A member of the TEC family kinases is thought to play a role in TSLP-mediated STAT5 activation, such as SOCS-1 (suppressors of cytokine signaling-1, an inhibitor of JAK and TEC kinases) which is able to inhibit TSLP-mediated signaling (Isaksen et al. 1999, 2002). Furthermore mTSLP receptor signaling requires STAT5, while mIL-7 receptor is also able to utilize STAT1 and STAT3 as well as STAT5 in its signaling (Isaksen et al. 1999; Lin et al. 1995; van der Plas et al. 1996).

Induction of TSLP Expression

TSLP expression can be triggered and enhanced by a variety of stimuli, so that a diverse array of signaling pathways may be involved in the induction of its expression (Fig. 1).

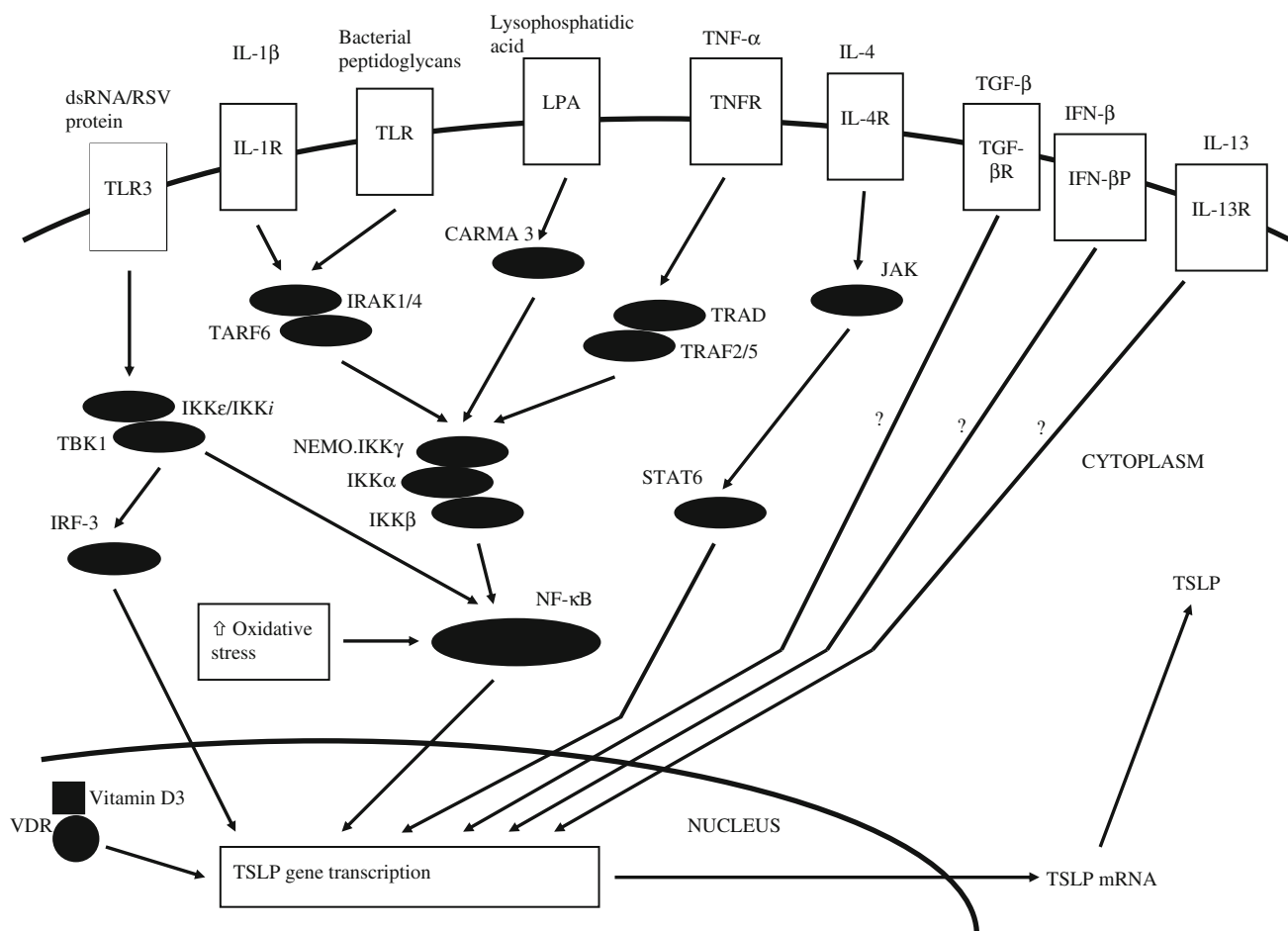


Fig. 1 The regulation of TSLP expression in epithelial cells. Viral dsRNA, viral protein, and bacterial peptidoglycans induce the expression of TSLP mRNA and protein via TLRs and the NF- κ B pathways. IL-1 β , lysophosphatidic acid, and TNF- α also use the NF- κ B pathway following activation of their corresponding receptors to induce the expression of TSLP mRNA and protein. IL-4, however, utilizes the JAK-STAT6 pathway to induce expression of both TSLP mRNA and protein. TGF- β , IFN- β , and IL-13 are able to induce the

expression of TSLP mRNA, although the pathway for this has yet to be elucidated and the increased expression of mRNA has not been accompanied by increased protein expression. It is also postulated that cigarette smoke and DEPs cause an increase in intracellular oxidative stress, which leads to NF- κ B activation and increased TSLP expression. In mice, vitamin D3, via its nuclear receptor VDR, has been shown to increase the expression of TSLP

1. Infection: viral infection in synergy with cytokine production is likely to be a primary driver for TSLP production. Rhinovirus and respiratory syncytial virus (RSV) can up-regulate the expression of TSLP in human bronchial epithelial cells (HBECs) (Tu et al. 2007). TSLP expression evoked by rhinoviral double-stranded ribonucleic acid (dsRNA) and RSV proteins acting on Toll-like receptors (TLR) is synergistically enhanced by IL-4, suggesting that nuclear factor- κ B (NF- κ B) and STATs cooperate in the transcriptional regulation of the TSLP gene (Allakhverdi et al. 2007; Kato et al. 2007). dsRNA synthesized by rhinoviruses is a ligand for TLR3. The induction of TSLP by dsRNA is dependent on TLR3, NF- κ B, and IRF-3, while enhancement of dsRNA-dependent TSLP expression by IL-4 is STAT6 dependent (Kato et al. 2007)
2. Cytokines and other mediators: pro-inflammatory cytokines (IL-1 β and tumor necrosis factor (TNF)- α), bacterial peptidoglycan, lysophosphatidic acid (LPA), and other TLR ligands (such as LTA, i.e. lipoteichoic acid, and poly I:C, i.e. polyinosinic:polycytidylic acid) are also able to induce the production of TSLP by HBECs (Allakhverdi et al. 2007; Lee and Ziegler 2007; Medoff et al. 2009). Additionally, the cytokines IL-4, IL-13, interferon (IFN)- β , and transforming growth factor (TGF)- β are able to up-regulate the expression of TSLP mRNA by HBECs, but have not been shown to be able to induce the production of TSLP at the protein level (Allakhverdi et al. 2007; Kato et al. 2007). Mutations in NF- κ B binding sites and 9-cis-retinoic acid (a NF- κ B inhibitor) have both been shown to inhibit the expression of TSLP

stimulated by IL-1 β in HBECs, indicating that pro-inflammatory cytokine induction of TSLP is regulated by NF- κ B in HBECs (Lee and Ziegler 2007; Lee et al. 2008). The induction of TSLP by LPA in HBECs is also mediated by NF- κ B and this activation of NF- κ B is dependent on CARMA3 (Medoff et al. 2009). This is further evidence to support the regulation of TSLP by NF- κ B in HBECs. IL-1 β and TNF- α can also induce the production of TSLP by human airway smooth muscle cells (HASMCS) independently and synergistically. Unlike HBECs, the expression of TSLP by IL-1 β and TNF- α is mediated via the ERK1/2 and p38 MAPK signaling pathways (Zhang et al. 2007). Mast cells were found to produce, store, and release TSLP following Fc ϵ RI aggregation. This was augmented in the presence of IL-4. TSLP released from mast cells was rapidly degraded not only by mast cell-derived proteases (tryptase, chymase, or both), but also by other undefined mediators (Okayama et al. 2009). In human skin keratinocytes, stimulation with IL-1 α or TNF- α in combination with Th2 cytokines (IL-4 or IL-13) is able to induce the production of TSLP in a synergistic manner (Okayama et al. 2009). Poly I:C, which mimics dsRNA and signals via TLR3, also induces the production of TSLP by human airway epithelial cells (Allakhverdi et al. 2007) and human keratinocytes. This induction of TSLP by dsRNA was enhanced by IL-4, IL-13 TNF- α , IFN- α , or IFN- β , while it was suppressed by IFN- γ , TGF- β , or IL-17 (Kinoshita et al. 2009).

3. Smoking and air pollution: the effects of cigarette smoking and pollution on TSLP production illustrate the importance of TSLP as a key cytokine at the interface between the environment and epithelium. Exposure of HBEC to diesel exhaust particles (DEPs) has been shown to up-regulate the expression of TSLP at both the mRNA and protein level. TSLP induction by DEPs was found to be mediated by an oxidant-induced pathway and was inhibited by increased free-radical scavenging by *N*-acetyl cysteine (Bleck et al. 2008). The action of DEPs is most likely mediated by NF- κ B as it is both involved in the regulation of TSLP production (Kato et al. 2007; Lee and Ziegler 2007; Lee et al. 2008; Medoff et al. 2009) and is known to be activated by oxidative stress in HBECs (Rahman 2000). Further indirect confirmation that oxidative stress may induce the release of TSLP can be found in our data showing that TSLP mRNA is elevated in the bronchial mucosa and the protein is elevated in the BAL fluid of patients with COPD (Ying et al. 2008). In a BALB/c ovalbumin mouse model of asthma, cigarette smoke extract has been shown to be able to induce the expression of TSLP mRNA and protein in

mouse lung. TSLP release was also inhibited by *N*-acetyl cysteine, highlighting the importance of the activation of TSLP via an oxidant-induced pathway (Nakamura et al. 2008).

The use of animal models has implicated several other cellular and nuclear pathways that may play an important role in the regulation of TSLP in humans. Keratinocytes from retinoid X receptor α and β knockout mice (RXR $\alpha\beta$ ^{ep-/-} mice) express high levels of TSLP and exhibit a skin and systemic syndrome similar to AD. 1 α ,25(OH) $_2$ D $_3$ (calcitriol), the physiologically active ligand of the vitamin D receptor (VDR), is able to stimulate murine keratinocytes to induce the expression of TSLP through binding of its nuclear receptor, VDR. Since the nuclear receptors RXR $\alpha\beta$ and unliganded VDR bind to form a heterodimer which acts as a co-repressor, binding of calcitriol to VDR or knockout of RXR $\alpha\beta$ will result in removal of this inhibition and TSLP expression by murine keratinocytes (Li et al. 2006). The peptidyl-prolyl isomerase PIN1, a regulator of phosphorylation signaling, catalytically regulates the conformation of substrates after their phosphorylation and so regulates protein function (Lu and Zhou 2007). In rats, PIN1 blockade reduces TSLP expression. PIN1 is thus likely to act as a potential modulator of TSLP expression at the post-transcriptional level (Esnault et al. 2008).

The Role of TSLP in DCs and T Cell Subtypes

TSLP strongly induces the expression of major histocompatibility complex class I and II as well as co-stimulatory molecules, such as CD40, CD80, and CD86, on DCs. It also prolongs DC survival and enhances DC-T cell conjugate formation, resulting in a strong proliferation of CD4⁺ and CD8⁺ T cells (Akamatsu et al. 2008; Gilliet et al. 2003; Soumelis et al. 2002; Watanabe et al. 2004). Unlike DCs, which express TSLPR, unstimulated CD4⁺ and CD8⁺ T cells do not express TSLPR and are not sensitive to TSLP. T cell receptor stimulation results in cell-surface expression of the TSLP receptor, allowing TSLP to act directly on activated CD4⁺ and CD8⁺ T cells, promoting proliferation (Akamatsu et al. 2008; Gilliet et al. 2003; Rochman et al. 2007).

In atopic dermatitis, TSLP expression by keratinocytes is associated with the activation and migration of Langerhans cells (LCs) from the epidermis to the dermis (Soumelis et al. 2002). TSLP enhances epidermal LC survival and migration and stimulates their production of thymus and activation-regulated chemokine (TARC) (Ebner et al. 2007). TSLP-activated DCs (TSLP-DCs) produce the chemokines IL-8, eotaxin-2, TARC, and macrophage-

derived chemokine (MDC) as well as the OX40 ligand (OX40L) (Isaksen et al. 2002; Ito et al. 2005; Soumelis et al. 2002). TSLP-DCs do not, however, produce the Th1-polarizing cytokine IL-12 or the proinflammatory cytokines TNF- α , IL-1 β , and IL-6 (Soumelis et al. 2002). TSLP-DCs also do not produce the Th2-polarizing cytokine IL-4 or the anti-inflammatory cytokine IL-10 (Soumelis et al. 2002). OX40L, a member of the TNF superfamily, has been implicated as the key cytokine by which TSLP-DCs polarize naïve CD4⁺ T cells to a pro-inflammatory Th2 response. Inhibition of OX40L by neutralizing antibodies results in strong inhibition of IL-4, IL-13, and TNF- α production while promoting the production of IFN- γ and IL-10 by CD4⁺ T cells primed by TSLP-DCs. This effect is not seen in CD4⁺ T cells primed by DCs stimulated by other ligands, such as TLR ligands and IFNs. The induction of Th2 response priming by TSLP-DCs can be inhibited and switched towards a Th1 response by the presence of exogenous IL-12, *Mycobacterium bovis* bacillus Calmette-Guérin (which down-regulates OX40L and stimulates IL-12 production), or simultaneous blockade of both OX40L (released by TSLP-DCs) and IL-4 (released by Th2 T cells) by neutralizing antibodies (Ito et al. 2005; Yokoi et al. 2008). On the other hand, TSLP-DCs induce the proliferation of naïve CD8⁺ T cells and induce CD8⁺ T cells to differentiate into cytotoxic CD8⁺ T cells that produce IL-5, IL-13, TNF- α , and IFN- γ (Gilliet et al. 2003).

T-regulatory (Treg) cells are involved in the regulation of the peripheral immune response and likely play a central role in determining the incidence and severity of several immune pathologies, including autoimmune disease, infectious disease, allergy, and asthma (Hawrylowicz and O'Garra 2005). The Treg subsets which have been identified are CD8⁺ Treg cells, natural killer (NK) cells, and CD4⁺ Treg cells (van der Plas et al. 1996). Naturally occurring Treg cells are CD4⁺CD25⁺Foxp3⁺ cells which originate in the thymus and are also possibly induced in the periphery (Hawrylowicz and O'Garra 2005). The epithelial cells of Hassall's corpuscles in the thymus express high levels of TSLP, which prime the DCs in the Hassall's corpuscles. These TSLP-DCs induce the proliferation and differentiation of CD4⁺CD8⁻CD25⁻ thymocytes into CD4⁺CD25⁺Foxp3⁺ T regs, which have suppressive functions similar to those of naturally occurring Tregs. This induction is dependent upon IL-2 and CD28 signaling (Watanabe et al. 2005).

The Role of TSLP in Other Cell Types

Mast cells play an important role in allergic inflammation, particularly in asthma, acting via multiple mechanisms.

Mast cells in patients with asthma produce Th2-type cytokines, induce IgE synthesis in B cells, up-regulate the production of a variety of cytokines/chemokines by epithelial cells and fibroblasts, and induce the recruitment of basophils, T cells, and eosinophils to sites of allergic inflammation as well as promote their own intraepithelial accumulation (Bradding et al. 2006; Hart 2001). Mast cells not only express TSLP mRNA (Ying et al. 2008), but are also a target cell for TSLP, expressing both TSLPR and IL-7R α (Allakhverdi et al. 2007). Stimulation of human mast cells in vitro with IL-1 β and TNF- α in the presence of TSLP strongly augments the production of the pro-inflammatory cytokines and chemokines while blocking endogenous TSLP released by activated primary human epithelial cells completely inhibits IL-1 β and TNF- α induced production of IL-13 by mast cells (Allakhverdi et al. 2007).

In the mouse, TSLP promotes an early stage of B-cell and T cell development (Levin et al. 1999; Sims et al. 2000). In contrast, in humans TSLP does not have direct effects on B cells, NK cells, or neutrophils (Liu et al. 2007).

TSLP Triggers AD and Asthma

Allergic diseases such as asthma and AD are characterized by their Th2 inflammatory profile with Th2 cytokine and chemokine release along with infiltration of their corresponding leukocyte targets. TSLP released by both bronchial epithelial cells and keratinocytes primes DCs to induce the expansion of naïve CD4⁺ T cells into pro-inflammatory CD4⁺ Th2 cells. This in turn drives and maintains the inflammation seen in these diseases (Bogiatzi et al. 2007; Ebner et al. 2007; Soumelis et al. 2002). The pro-inflammatory cytokines released by TSLP-DC-primed T cells are characteristic of asthma and AD which are possibly mediated by IL-4, IL-13, and TNF- α , but not by IL-10 or IFN- γ (Ebner et al. 2007; Soumelis et al. 2002).

The effects of TSLP in vitro on DCs and T cells are mirrored by the in vivo findings in disease. In AD, the production of TSLP is restricted to keratinocytes of the apical layers and seems to be associated with the migration of activated LCs from the epidermis to the dermis (Bogiatzi et al. 2007; Soumelis et al. 2002), although the mechanisms of the migration are unclear. In asthma we have found that TSLP expression is increased in asthmatic airways and correlates with the expression of the Th2-attracting chemokines TARC/CCL17 and MDC/CCL22 in endobronchial biopsies and with disease severity. We found that epithelial cells, endothelial cells, neutrophils, macrophages, and mast cells expressed TSLP (Ying et al. 2005). Further evidence of the key role that TSLP plays in asthma

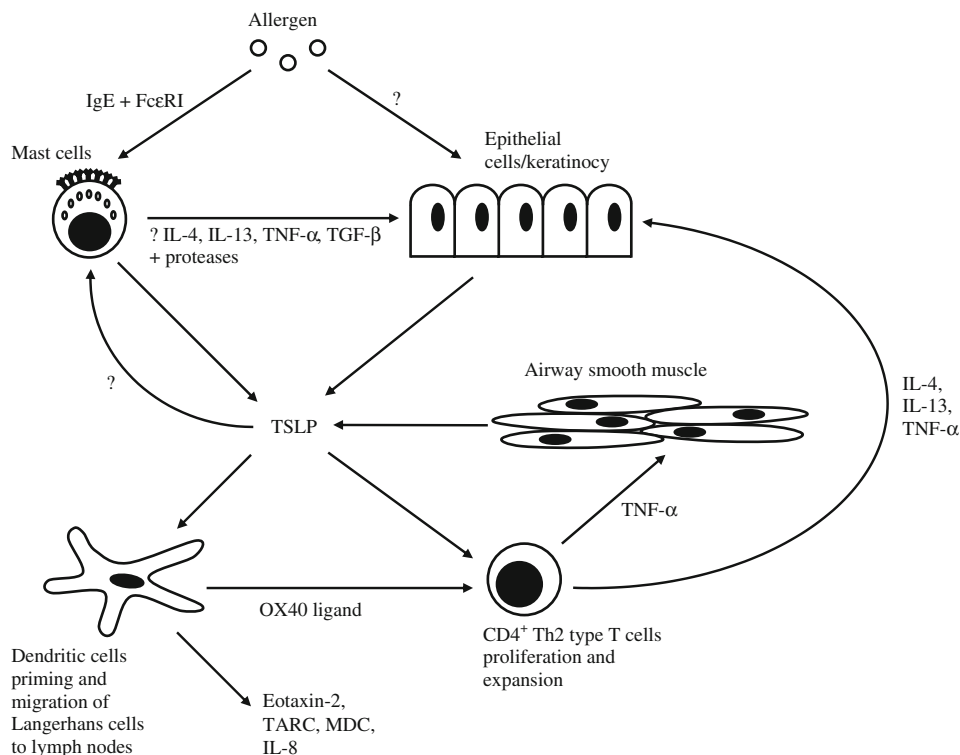


Fig. 2 The expression and function of TSLP in asthma and atopic dermatitis (AD). The expression of TSLP in asthma and AD is allergen driven. Allergen activates mast cells via IgE and FcεRI. This causes the mast cell to release TSLP, various inflammatory cytokines, and other mediators such as proteases. These inflammatory cytokines are likely to be the signal that causes epithelial cells/keratinocytes to produce TSLP. It is not known if allergen can directly act on epithelial cells/keratinocytes to induce the production of TSLP. The TSLP release primes DCs to secrete Th2-attracting chemokines as well as OX40 ligand. TSLP also promotes a subtype of DCs,

Langerhans cells, to migrate from the site of induction to the lymph nodes. OX40 ligand is one of the signals required from DCs to prime naïve T cells to differentiate into CD4⁺ Th2-type T cells and enhance their proliferation and expansion. TSLP is also able to act on activated T cells to enhance proliferation and expansion. Th2 cytokines released by T cells feedback positively to the epithelial cells and stimulate TSLP production. TNF-α also stimulates airway smooth muscle to release TSLP, therefore creating another positive-feedback loop

and AD is available from animal “models” of these diseases. For example, transgenic mice that have inducible TSLP over-expression in keratinocytes develop an AD-like phenotype following TSLP induction characterized by eczematous skin lesions containing inflammatory cell infiltrates, a dramatic increase in circulating Th2 cells, and elevated serum IgE (Yoo et al. 2005). Transgenic mice that over-expressed TSLP in the lungs had an augmented Th2 inflammatory response (infiltration of leukocytes and release of both Th2 cytokines and IgE) and remodeling (goblet cell hyperplasia and subepithelial fibrosis) when challenged with ovalbumin. Conversely, TSLP receptor-deficient mice (TSLPR knockout) showed an attenuated Th2 inflammatory response and remodeling when challenged with ovalbumin (Zhou et al. 2005). Murine models have also shown that the Th2 inflammatory response induced by TSLP is T cell and eosinophil dependent (Jessup et al. 2008). Furthermore, the response to inhaled antigen can be restored in TSLPR knock-out mice by transfer of CD4⁺ T cells from wild-type mice into the

knock-out mice (Al-Shami et al. 2005). Attenuation of the Th2 inflammatory response and remodeling in this ovalbumin murine “model” of asthma can also be achieved by administration of anti-TSLPR antibodies prior to ovalbumin sensitization (Shi et al. 2008), suggesting that this blockade might reduce various functions of TSLPR⁺ cells involved in the pathogenesis of asthma. The potential role of TSLP in asthma is summarized in Fig. 2.

The Role of TSLP in COPD

COPD is a disease characterized by inflammation of the central and peripheral airways which, as with asthma, has been circumstantially implicated in contributing to airway obstruction but, in contrast to asthma, is poorly responsive to anti-inflammatory therapy such as corticosteroid. A second key mechanism in COPD is destruction of the lung parenchyma, reducing elastic support for the small airways, which causes them to collapse, and eliminating alveoli,

thus impairing gas transfer (Di Stefano et al. 2004). Cigarette smoking is a major risk factor for the development of COPD, with over 90% of COPD patients in the developed world being cigarette smokers (Pauwels et al. 2001; Siafakas et al. 1995). The cellular inflammation in COPD is characterized by increased numbers of neutrophils, macrophages, and T lymphocytes, with a preponderance of CD8⁺ (cytotoxic) over CD4⁺ (helper) cells in the bronchial mucosa and increased macrophages and neutrophils in BAL fluid and induced sputum (Di Stefano et al. 1998; Keatings et al. 1996; Pesci et al. 1998; Saetta et al. 1998; Sarir et al. 2008). The key inflammatory mediators in COPD are thought to be the cytokine TNF- α and the chemokine IL-8 (Reimold 2002; Sarir et al. 2008; Weathington et al. 2006). IFN- γ and IL-13 have also been implicated in causing emphysema, at least in animal “models” (Elias et al. 2006).

There is considerable evidence that increased oxidative stress plays an important role in the pathogenesis of COPD. Cigarette smoke, which is rich in oxidative substances, increases the oxidative burden in the lungs, compounding the innate oxidative stress created in inflammatory processes through the generation of reactive oxygen and other

species by inflammatory, immune, and epithelial cells in the airways, leading to further inflammation and tissue destruction (Elias et al. 2006; Rahman and MacNee 1996; Repine et al. 1997). Furthermore NF- κ B, which regulates the release of many cytokines and chemokines, is responsive to the oxidative stress (Rahman 2000). We postulate that the activation of NF- κ B by oxidative stress from cigarette smoke contributes to elevated TSLP production in the bronchial mucosa in COPD (Kato et al. 2007; Lee and Ziegler 2007; Lee et al. 2008; Medoff et al. 2009; Rahman 2000; Ying et al. 2008). We discovered elevated bronchial mucosal expression of mRNA encoding TSLP and the chemokines TARC/CCL17, MDC/CCL22, and IFN-inducible protein 10/CXCL10, with corresponding elevation of these proteins in the BAL fluid in COPD (Ying et al. 2008). We also found a similar pattern of expression at the protein level (although there was low level of TSLP) in smokers without COPD (Ying et al. 2008). These data are consistent with the hypothesis that this expression is most likely due at least partly to the effects of cigarette smoke on TSLP expression and the corresponding effects of TSLP on DC release of chemokines in COPD. The fact that not all smokers develop COPD suggests that there are likely

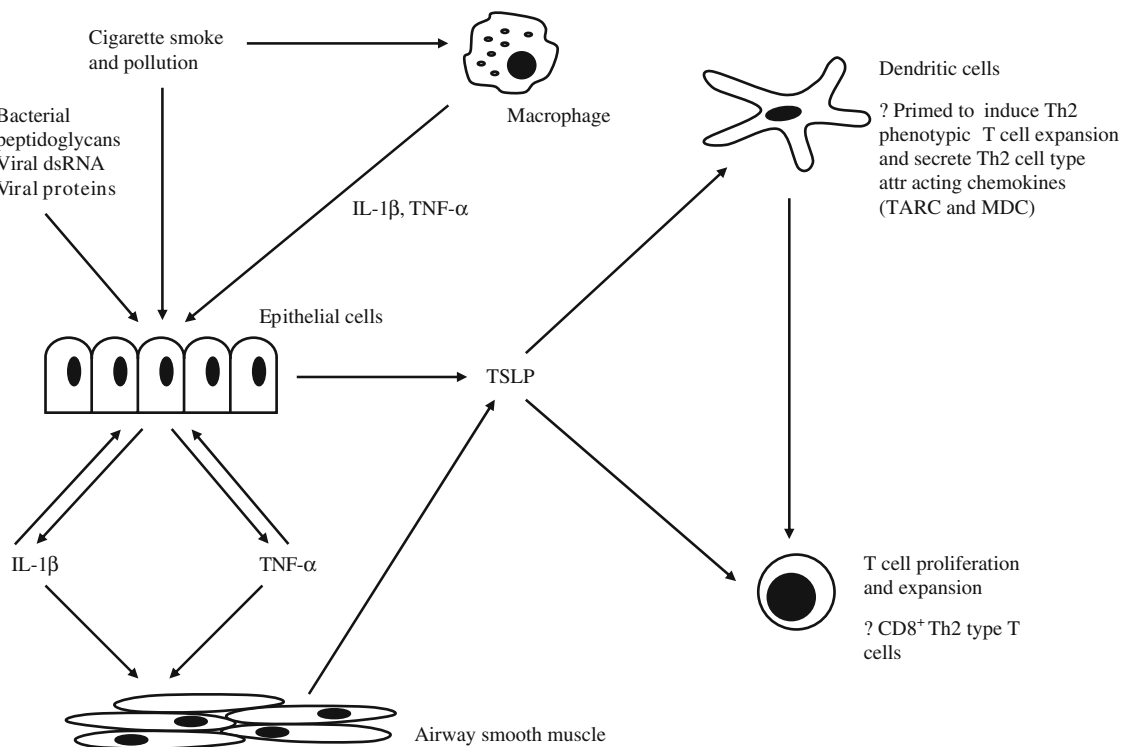


Fig. 3 The expression and function of TSLP in COPD. The expression of TSLP in COPD is most likely multi-factorial, with oxidative stress from cigarette smoke and pollution, bacterial, and viral infections as well as inflammatory mediators released from macrophages stimulated directly by cigarette smoke and pollution. Cigarette smoke and pollution also directly stimulate epithelial cells

to produce IL-1 β and TNF- α , which could act in an autocrine fashion stimulating the release of TSLP. The exact function of TSLP in COPD is yet to be determined. TSLP could possibly prime DCs to induce the secretion of Th2-attracting chemokines and induce cytotoxic CD8⁺ Th2-type T cell proliferation and expansion

additional factors which drive the development of COPD in particular individuals. Oxidative stress is most likely not the only mechanism by which TSLP expression is elevated in COPD (Fig. 3). The cytokines TNF- α and IL-1 β , which are associated with COPD, may also play a key role in the elevation of TSLP expression (Sarir et al. 2008). Each of these cytokines is able to induce the expression of TSLP by HBECs and HASMCs (Allakhverdi et al. 2007; Lee and Ziegler 2007; Zhang et al. 2007). Another mechanism that may play a role in the induction of TSLP in COPD is chronic infection. There is increasing evidence for persisting bacterial or viral infection in stable COPD (Miyata et al. 2008; Sethi and Murphy 2008). Again, bacterial peptidoglycan and TLR ligands (LTA, dsRNA, RSV proteins) have been shown to be able to induce HBECs to produce TSLP (Allakhverdi et al. 2007; Kato et al. 2007; Tu et al. 2007) (Fig. 1). As we found a significant elevation of TSLP protein in the BAL fluid of COPD patients but not smokers without COPD, it is possible that these cytokines (TNF- α and IL-1 β), persistent viral/bacterial infection, and oxidative stress all act synergistically to enhance the production and expression of TSLP in COPD (Ying et al. 2008).

TSLP is able to promote the expansion of both CD4⁺ and CD8⁺ T cells either directly following activation or indirectly via DCs. In vitro and in vivo studies have suggested that TSLP primes the T cell response towards a Th2-type inflammatory response (Akamatsu et al. 2008; Gilliet et al. 2003; Rochman et al. 2007; Watanabe et al. 2004). While TSLP may contribute to expansion of CD8⁺ T cells, of which there is a predominance in COPD (Di Stefano et al. 2004; Saetta et al. 1998; Sarir et al. 2008), it is as yet not clear why, despite elevated expression of TSLP and Th2 chemokines in COPD, there does not appear to be a Th2 cellular response and corresponding cytokine response. It is alternatively possible that TSLP is induced nonspecifically in certain inflammatory processes in which it does not play a key functional role.

Conclusion

TSLP is a cytokine that is highly expressed by skin keratinocytes in AD and bronchial epithelial cells in asthma and COPD. A variety of stimuli can induce its expression, including bacterial and viral infection, oxidant stress, and the local cytokine milieu. Activation of NF- κ B plays a key role in the expression of TSLP in these diseases. However, there are most likely other signaling pathways involved in TSLP expression that have yet to be discovered. These may interact in various ways to regulate the expression of TSLP mRNA and protein in different circumstances. The precise mechanism which results in the

production of TSLP by structural cells following exposure to allergen has yet to be determined in humans. The activation of mast cells by allergen and the release of its inflammatory products (TNF- α , IL-4, and IL-13) (Bradding et al. 2006; Hart 2001) is one possible mechanism. A murine model of allergic rhinitis supports this theory, as mast cell-deficient or Fc receptor γ chain-deficient mice show attenuated TSLP expression in epithelial cells in the response to allergen (Miyata et al. 2008). The evidence is clear that TSLP not only initiates, but also enhances and propagates the Th2 inflammatory response in allergic inflammation. Although it is clear that the signaling pathway of TSLPR involves STAT5 activation, the mechanism by which this occurs is unknown, as JAK activation is not apparently initiated by the TSLPR. The most intriguing finding is that TSLP is elevated in COPD. The mechanism by which this occurs and the roles which TSLP plays in COPD need further investigation.

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