

UNSOLICITED REVIEW

Function and mechanisms of TSLP/TSLPR complex in asthma and COPD

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Abstract

Thymic stromal lymphopoietin (TSLP) is a key pro-allergic cytokine that has recently been linked to chronic airway diseases, such as asthma and chronic obstructive pulmonary disease (COPD). High levels of TSLP were detected in bronchial mucosa of asthma and COPD patients suggesting TSLP's biological role beyond a signature 'Th2-favoring' or 'pro-allergic cytokine'. Besides inflammatory cells, airway structural cells produce and are targets of TSLP suggesting a potential autocrine loop that may have a profound effect on local inflammatory response and airway remodelling. This review sums up diverse mechanisms that mediate TSLP/TSLP receptor-signalling network in chronic airway diseases.

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Introduction

Global burden of chronic airway diseases, such as asthma and chronic obstructive pulmonary disease (COPD), is increasing gradually. Asthma is a chronic disorder of the airways in which the inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing. It is among the commonest chronic conditions in Western countries affecting approximately 14% children and 8% adults. With an estimated global burden of 300 million people, asthma is responsible each year for 1500 avoidable deaths, as well as 20 million lost working days [1, 2].

On the other hand, COPD, as defined by the Global Obstructive Lung Disease (GOLD) guidelines, is a preventable and treatable disease with significant extra-pulmonary effects that may contribute to the severity in individual patients. The pulmonary component is characterized by airflow limitation that is not fully reversible either spontaneously or after treatment. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases [3]. The COPD prevalence, as defined using GOLD criteria of stage 2 or higher dis-

ease, ranges from 3% to 23% of the population across different countries [4].

Cumulative evidence suggests that the pathologies of both asthma and COPD occur due to an abnormal immune response. Traditionally, chronic immune inflammation is believed to trigger and perpetuate a vicious circle of tissue 'injury and repair' culminating in tissue remodelling. The focus of the research for many years has been on lung-infiltrating immune cells and the array of locally released pro-inflammatory mediators called cytokines [5, 6]. The role of T helper, (Th)1 and Th2 cells and associated cytokines was suggested by animal models and confirmed in human studies [7, 8]. However, later studies did not support this hypothesis as the proposed cytokines (IL-4, IL-5 and IL-13) did not correlate with the disease severity in humans, eventually putting in question the role of T cells in these pathologies [8–11]. Eventually, the focus started getting shifted towards the local tissue components [12]. Although the role of T cells and Th cytokines (Th1, Th2, Th17, and Treg) is certainly an active area of investigation (reviewed in [13, 14]), structural components have emerged as key players in instigating local remodelling and inflammation. In fact, the data from paediatric severe asthma showing early presence

of structural alterations in bronchial tissue suggest that inflammation and remodelling may occur in parallel and also that the latter may arise independent of former [12, 15, 16]. Interestingly, the rhesus monkey model also suggests that airway wall remodelling can occur long before any signs of inflammation; particularly, the smooth muscle was found to play a special role in commencing the attraction and activation of infiltrating immune cells [17–19].

Among other components, airway smooth muscle (ASM) cells have emerged as crucial players in regulating the airway tissue homeostasis, and remodelling associated with chronic diseases. An increased ASM mass caused by hypertrophy and hyperplasia is a cardinal feature of both asthma and COPD. Airway wall remodelling occurs in the upper airways in asthma and in the distant airways in COPD [20, 21]. The ASM cells can contribute directly to the pathogenesis of asthma by expressing cell adhesion and costimulatory molecules and by secreting multiple proinflammatory cytokines and chemokines that may perpetuate airway inflammation and the development of airway remodelling [22, 23]. Convincing evidence suggests that ASM cells might be compared to, and indeed classified as, 'inflammatory-like' cells by virtue of their capacity to produce and respond to multiple pro-inflammatory mediators [22–24]. Indeed, it is now evident that ASM can directly respond to inhaled environmental factors via mechanisms that are either immune-mediated, such as both high- and low-affinity IgE receptors [25–27], or in an immune-independent fashion, such as protease activated receptor-2 (PAR2) or calreticulin, summarized in [8]. Therefore, understanding the ASM behaviour is essential to fully appreciate the role it plays in chronic airway inflammatory diseases.

This review focuses on a newly discovered, hematopoietic-like proallergic cytokine thymic stromal lymphopoietin (TSLP) which has been demonstrated recently to be produced by ASM in health and disease. We discuss herein the emerging biological role of TSLP in modulating ASM function in relationship with airway inflammation, bronchoconstriction and remodelling in asthma and COPD.

Thymic stromal lymphopoietin and its receptor

The TSLP is a novel interleukin 7 (IL-7)-like cytokine, originally isolated from a murine thymic stromal cell line [28] and characterized as a lymphocyte growth factor [29]. The human TSLP gene is located on chromosome 5q22.1 next to the atopic cytokine cluster on 5q31 [30]. Both human and mouse TSLP exert their biological activities by binding to a high-affinity TSLP receptor (TSLPR) complex that is a heterodimer of TSLPR chain which is closely related to the common

receptor γ chain (γc) and interleukin 7 receptor- α (IL-7R α) [31, 32]. Although the sequence homology for both TSLP and its receptor between mouse and human is only about 40%, their biological functions appear to be similar [33].

Albeit initially thought to be a thymic epithelial cell-derived cytokine, TSLP is now known to be produced by epithelial cells in the lungs, gut and skin, fibroblasts, ASM, endothelial cells, mast cells, macrophages, granulocytes [34–39], synovial fibroblasts, intervertebral disc cells [40] and more recently, by dendritic cells (DCs) [41]. The expression of TSLP is regulated by several factors, including both exogenous stimuli, including trauma, mechanical injury, infection with microbes, Toll-like receptor (TLR) ligation, and host-derived pro-inflammatory and Th2 cytokines [42, 43]. The TSLP-stimulated myeloid DCs (mDCs) can prime naïve CD4⁺ T cells in an antigen-specific manner, resulting in T cells showing characteristic features of Th2-differentiated inflammatory cells (i.e. production of IL-4, IL-5, IL-13 and TNF), with the exception of IL-10 production [44]. Interestingly, in the absence of IL-12, TSLP induces the expression of OX40L, the ligand for cell survival factor OX40 on DCs; OX40-OX40L interactions were found to be critical for the ability of the mDCs to drive Th2 cell differentiation [45]. Besides activating mDCs and inducing proallergic CD4⁺ or CD8⁺ responses [44, 46], TSLP can also synergize with IL-1 β and TNF- α to induce Th-2 cytokine and chemokine (IL-5, IL-13, IL-6, GM-CSF, CXCL8 and CCL1) expression in mast cells [35]. Interestingly, TSLP was found to be increased in synovial fluid specimens derived from patients with rheumatoid arthritis (RA), suggesting that TSLP might even play a role in autoimmune diseases, such as RA [47]. Recent data also suggest that TSLP aggravates the inflammation and disease progression in pancreatic and breast tumours, by promoting Th2 cell recruitment and creating a tumour-conducive environment [48, 49].

The expression of TSLPR has been detected on mDCs, CD4⁺ and CD8⁺ T cells, B cells, mast cells, natural killer T (NKT) cells and monocytes as well as in tissues from heart, skeletal muscle, kidney and liver [42]. This suggests that TSLP can act on a broad range of cell types, notably both innate and adaptive immune cells. In contrast, emerging reports suggest that the TSLP serves as a potent homeostatic regulatory cytokine, such as the TSLP expression in normal pregnancy trophoblasts with significant Th2 bias is much higher than that of miscarriage showing Th1 bias at the maternal–fetal interface [50]. Altogether, the precise biological functions of TSLP in health and disease remain yet to be established, and therefore deserve intensive investigation.

Thymic stromal lymphopoietin expression in airway smooth muscle

Given the emerging role of TSLP in allergic inflammatory diseases [44–46] and the ‘inflammatory-like’ nature of ASM [22–24], it was enticing to assess whether ASM could contribute to airway inflammatory response via production of, and/or responsiveness to TSLP. We showed in a very first report, the constitutive and *in vivo* expression of TSLP in ASM of COPD patients [38]. Pro-inflammatory cytokines TNF- α , and IL-1 β augmented the ASM TSLP expression, via p38 and Erk1/2 mitogen-activated protein kinase (MAPK) pathways activation. It was also demonstrated recently that the NF- κ B, and AP-1-like transcription factors are critical in TNF-induced TSLP release in ASM cells (Fig. 1) [51]. The data from our group also suggest an IgE-inducible TSLP expression in ASM involving Syk and NF- κ B pathways [52]. However, in contrast to airway epithelial cells [36], stimulation of ASM with LPS (TLR 4 agonist) and cytosine phosphate guanine (CpG) (TLR 9 agonist) did not induce TSLP release, even though ASM cells express TLR 2, 3, 4 and 9 [53, 54]; suggesting perhaps different signalling mechanisms in ASM compared to the epithelial cells. Allakhverdi et al. [34] corroborated these findings by showing TNF- α , and IL-1 β -inducible TSLP expression in non-asthmatic ASM cells (Fig. 1). Interestingly, given the close proximity of ASM with mast cells in airways [55], authors also examined whether TSLP could serve as a mediator of cross-talk

between these two cell types. Indeed, supernatants of IgE/anti-IgE-activated mast cells induced the TSLP release in ASM cells [34]. Notably, neither additional Th1 (IFN- γ) nor Th2 (IL-4, IL-9) cytokines induced the TSLP expression in ASM [34, 38]. Except TNF- α , other mast cell mediators (e.g. tryptase, histamine, leukotriene C₄, prostaglandin E₂ and prostaglandin D₂) could not induce TSLP release in ASM cells [34]. Interestingly, the culture supernatants of IL-1 and TNF-stimulated ASM cells triggered the release of IL-5 and IL-13 by mast cells, the response that was completely inhibited by anti-TSLP antibody, suggesting a TSLP-dependent interaction [34]. Collectively, this study suggested a TSLP-mediated potential cross-talk between ASM and mast cells.

Furthermore, Futamura et al. [56] showed the TNF- α -mediated induction of TSLP release in human ASM cells, normal bronchial epithelial cells (NBEC) and normal human lung fibroblasts (NHLF). Whereas IL-4 alone failed to induce TSLP, it synergized with TNF- α to produce TSLP in all three cell types (ASM, NBEC and NHLF). One surprising observation from this study [56] was that the airway mesenchymal cells (ASM and NHLF) produced higher amounts of TSLP compared to NHBE. This may underline the contribution of ASM in modulating airway immune response in an epithelial cell-independent manner. Finally, a recent study demonstrated that 1–2% cigarette smoke extract (CSE) exposure augments the basal TSLP expression in ASM cells [57], adding up towards a pathogenic role of CSE

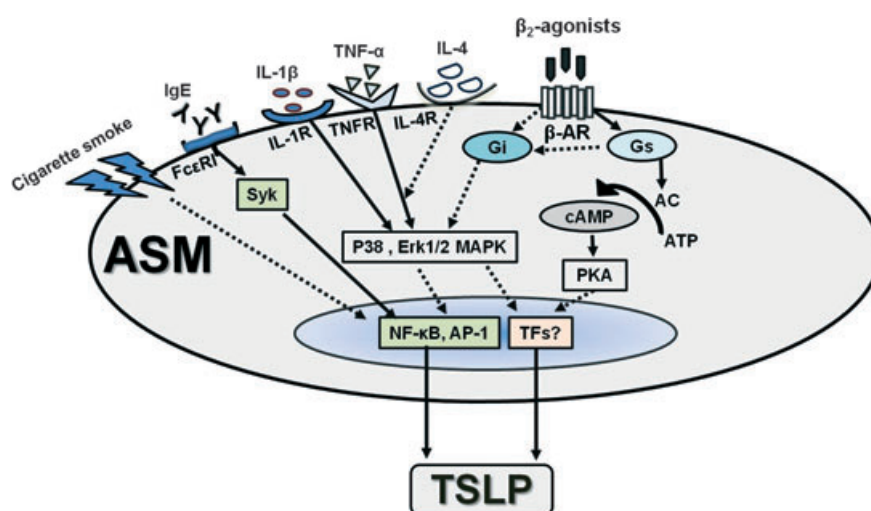


Fig. 1. Triggers and mechanisms of thymic stromal lymphopoietin (TSLP) expression in airway smooth muscle (ASM). Pro-inflammatory (IL-1 β , TNF- α) cytokines induce TSLP in ASM via MAPK, NF- κ B and AP-1; whereas IgE utilizes Syk, NF- κ B and AP-1 pathways. Th2 cytokine IL-4 synergizes with TNF- α , cigarette smoke extract induces TSLP, whilst β 2-agonists were recently shown to augment the cytokine-induced TSLP expression in ASM cells. Although mechanisms remain obscure, β 2-agonists may involve the G-protein-coupled receptor (GPCR)'s Gi subunit stimulation that induces Erk activation and eventually synergize with cytokine-induced Erk MAPK pathways. This might also involve unknown transcription factors that are activated through cAMP-PKA pathway. Solid line arrows indicate known pathway(s) and dotted line arrows represent plausible pathway(s). β -AR, β -adrenergic receptor; Gs, stimulatory GPCR subunit; Gi, inhibitory GPCR subunit; AC, adenylate cyclase; PKA, protein kinase A, TFs, transcription factors.

in airway inflammation. Authors found that this effect was neither due to nicotine nor hydrogen peroxide (H_2O_2), arguably two major biologically active constituents of CSE [57]. However, pre-exposure of ASM with the reactive oxygen species (ROS) scavenger *N*-acetylcysteine (NAC) abrogated the CSE-induced increase in TSLP expression, suggesting at least partial involvement of oxidative stress [57]. In conclusion, these studies [34, 38, 56, 57] provide convincing evidence for ASM to be a rich source of TSLP (Fig. 1) that may modulate the airway inflammatory response.

β_2 -agonists modulate the thymic stromal lymphopoietin expression in airway smooth muscle

Beta 2 (β_2)-adrenergic receptor (β_2 -AR) agonists (commonly called β_2 -agonists) are widely used as bronchodilators in the treatment of asthma owing to their potent bronchodilatory effects on ASM. In addition, they may exert anti-inflammatory effects, including inhibition of granulocyte functions [56, 58]. However, a number of clinical studies have recently found that the regular use of β_2 -agonist alone has adverse effects on asthma control, and pose an increased risk of asthma death [59, 60]. Some studies highlight the potential of β_2 -agonists to enhance Th2 cytokine-mediated inflammation [61]. Indeed, it was recently reported that the cytokine (IL-4 plus TNF)-induced TSLP production by lung mesenchymal (ASM and fibroblasts) and epithelial cells was enhanced by β_2 -agonists of either long-acting (salmeterol, formoterol) or short-acting (salbutamol) nature [56] (Fig. 1).

Known mechanisms of action of β_2 -agonists on ASM involve the activation of G_s - α receptor subunit, which stimulates adenylate cyclase to catalyse the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). The cAMP induces its relaxing and other effects on ASM predominantly through the activation of protein kinase A (PKA) [61, 62]. Interestingly, the treatment of ASM cells with cAMP elevating agents (e.g. db cAMP, 8-Br cAMP and forskolin) augmented the TSLP production [56]. Therefore, although the effect of β_2 -agonists on TSLP release in ASM appears to directly involve cAMP-PKA pathway, there may be additional mechanisms involved as well, such as the exchange protein directly activated by cAMP (Epac) [63]. For instance, the use of PKA inhibitors could not modulate the β_2 -agonist-enhanced TSLP production [56].

Some studies provide mechanistic insights into the adverse effects associated with chronic use of β_2 -agonist monotherapy in asthma. One possible explanation for these effects (e.g. bronchoconstriction) is the β_2 -AR desensitization due to chronic and strong β_2 -agonist stimulation [64, 65]. Second, the persistent high-level

activation of β_2 -AR can increase the expression of phospholipase C- β (PLC- β) in mouse ASM, which leads to an increase in intracellular calcium, eventually leading to the activation of myosin light chain kinase (MLCK), myosin phosphorylation and consequent muscle contraction [66]. Third, it has been demonstrated that β_2 -AR can also couple to the G_i (inhibitory) proteins, resulting in phosphorylation of the Erk and p38 mitogen-activated protein kinase (MAPK) pathways [67]. Moreover, G_s -to- G_i switching could be exaggerated by inflammatory cytokines (IL-1 β) that have been shown to increase G_i expression [68], eventually facilitating Erk activation. In light of these findings [67–69] and our previous report that cytokine-induced TSLP requires Erk1/2 and p38 MAPK activation [38], it is conceivable that β_2 -agonists and proinflammatory cytokines (IL-1 β , TNF- α) work in a concerted fashion to induce TSLP production in ASM cells by activating MAPK pathways (Fig. 1) [38, 56]. Collectively, this study [56] offers yet another mechanism, albeit with meagre details, of how β_2 -agonist may contribute to Th2 responses, potentially by elevating TSLP production.

According to the recently updated guidelines for asthma management [70], the preferred treatment regimen for patients with intermittent asthma is an inhaled short-acting β_2 -agonist, in combination with an inhaled corticosteroid. Authors in current study [56] in fact found that a corticosteroid, fluticasone significantly inhibits the TSLP production by all lung tissue cell types (NHBE, ASM and NHLF). Interestingly, glucocorticoids (GC) were recently shown to inhibit dsRNA-induced TSLP expression in skin keratinocytes [71]. Although mechanisms remain yet to be fully established, the study by Futamura et al. [56] advocates the combination therapy with a β_2 -agonist and corticosteroid to effectively inhibit the TSLP-mediated lung inflammation.

Thymic stromal lymphopoietin in chronic obstructive pulmonary disease: A Th2 cytokine in Th1 disease

The COPD is a major cause of morbidity and mortality world-wide [4, 72]. 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 [73, 74]. The key inflammatory mediators suggested in COPD are the cytokines TNF- α , IL-1 β and the chemokine IL-8/CXCL8 [75]. With emerging evidence for TSLP in inflammatory diseases, an enhanced TSLP expression was observed in ASM bundles of COPD patients [38]. In COPD, although many studies have reported a preponderance of Th-1 cytokines, there are a number of studies showing Th2-biased response in the airways, especially in subjects who fulfil the criteria for

diagnosis of chronic bronchitis [76]. This Th2 response appears to be modulated by CD8 + T cells. Interestingly, human TSLP primes dendritic cells to induce naïve CD8 + T cell activation and differentiation into IL-5-producing cells [46]. In light of these studies and our findings, it is likely that TSLP expression by human ASM cells may influence immune regulation by interacting with and influencing local immune cells in COPD airways.

Ying et al. [37] later showed an elevated mRNA expression of TSLP, and chemokines TARC/CCL17, MDC/CCL22 and IP-10/CXCL10 in bronchial mucosa, with corresponding elevation of these proteins in the BAL fluid of COPD patients. Considerable evidence suggests that increased oxidative stress can also be critical in the pathogenesis of COPD. Notably, the cigarette smoke (rich in oxidative substances) increases the oxidative burden in the lungs, amplifying the inherent oxidative stress caused through the generation of reactive oxygen species by inflammatory immune, and epithelial cells in the airways, eventually causing enhanced inflammation and tissue injury [74, 77]. Furthermore, NF- κ B, which regulates the release of many cytokines and chemokines, is shown to be responsive to the oxidative stress [78]. It was proposed recently [74] that the activation of NF- κ B by oxidative stress from cigarette smoke may underlie the elevated TSLP production in the bronchial mucosa in COPD [36, 37, 78–80]. Furthermore, repeated intranasal exposure to CSE was found to increase the lung TSLP expression, predominantly through oxidative stress and TNFR activation [81], linking CSE and TSLP expression in lung for the first time. Interestingly, Smelter et al. report that the chronic CSE exposure augments the TSLP expression in ASM cells [57], providing at least one direct target of CSE in inducing the TSLP in lungs. Finally, Rhinovirus (RV16) or dsRNA was shown to induce TSLP expression in COPD epithelium in a TLR3-dependent manner [82]. Altogether, it is plausible that the pro-inflammatory cytokines (TNF- α , IL-1 β), bacterial/viral infections and oxidative stress from cigarette smoke contribute synergistically towards elevated TSLP expression in BAL fluid of COPD patients [37]. Collectively, it is indeed surprising to find elevated TSLP expression in COPD, which is conventionally thought to be a 'Th-1-dominated' inflammatory disease, and therefore needs intensive investigation that can demarcate the exact immunoregulatory role of TSLP in this airway pathology.

Thymic stromal lymphopoietin in asthma

The TSLP was found to be necessary and sufficient to drive Th2 cytokine-mediated airway inflammation in murine models of asthma. Lung-specific expression of a TSLP transgene induced allergic airway inflammation characterized by a massive infiltration of inflammatory

cells, goblet cell hyperplasia and subepithelial fibrosis, with increased serum IgE levels [83]. On the contrary, mice lacking the TSLPR failed to develop asthma in response to inhaled antigen (ovalbumin plus alum), probably due to the inability of CD4 + T cells to respond to TSLP, as reconstitution with TSLPR-positive T cells restores the aspects of the inflammatory disease [84]. Interestingly, a recent study implicates that TSLP functions to condition the local environment to respond to aerosolized antigens. Direct intranasal delivery of TSLP and antigen led to rapid onset of severe disease. The disease in these mice develops slowly over a 3-month period, as determined on the basis of adaptive responses to environmental antigens. However, antigen challenge at an early age (in presence of TSLP) leads to the immediate onset of disease [85]. The findings from these animal models are closely dictated in human asthmatic subjects where higher concentrations of TSLP have been detected in the lungs, correlating with Th2 attracting chemokines and disease severity [37, 39]. Indeed, an IgE-induced TSLP expression in ASM clearly points towards a vicious cycle of allergic airway inflammation mediated by TSLP [52]. Furthermore, *in vivo* immunoreactivity of TSLPR on ASM bundles in mild allergic asthma patients [86] hints a previously undefined role of ASM in asthma pathogenesis in a TSLPR-dependent manner, plausibly through the recruitment of inflammatory immune cells in airways (Fig. 2).

Although it is not very clear, emerging evidence suggests a potential role of TSLP in 'atopic march', the phenomenon in which people with one atopic disease (e.g. AD) are more likely to develop a second or third (allergic rhinitis/asthma) [87]. Finally, genetic analysis of atopic populations has shown an association of TSLP gene polymorphisms with features of allergic disease, including asthma and airway hyperresponsiveness, high IgE concentrations and eosinophilia [88–91].

Thymic stromal lymphopoietin/TSLPR-mediated airway smooth muscle activation: potential autocrine loop?

In context of the global hypothesis of ASM's role as a critical airway immunomodulatory cell [22–24], numerous studies have implicated the pro-inflammatory and Th-2 cytokines/chemokines and their receptors in ASM cell activation. For instance, ASM cells are a rich source of IL-8/CXCL8 [92] and express the IL-8/CXCL8 receptors (CXCR1, and CXCR2), activation of which leads to increase in intracellular Ca²⁺ concentration ([Ca²⁺]_i), cell contraction and cell migration [93]. Similarly, ASM cells produce and respond to eotaxin-1/CCL11 [94], and IL-6 [95]. Therefore, the ASM-produced TSLP may either be involved in regulation of local airway immune response, for example, by targeting mast cells that are

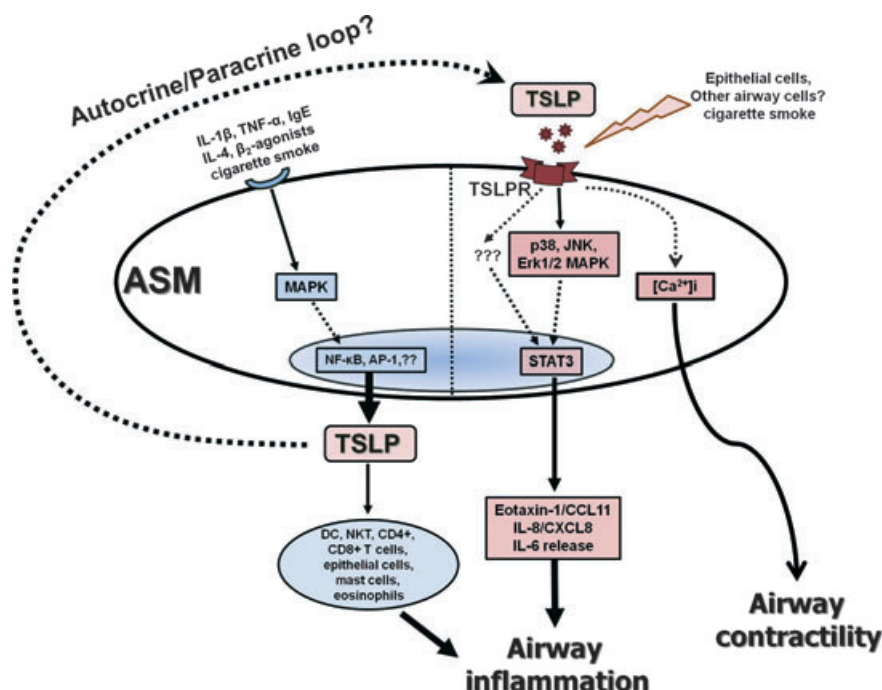


Fig. 2. Thymic stromal lymphopoietin (TSLP)/TSLPR-mediated autocrine activation of airway smooth muscle ASM. Cytokines-, β_2 -agonists- or cigarette smoke-induced TSLP from ASM may act on airway structural or lymphoid/myeloid cell types eventually contributing to airway inflammation. A functional TSLPR presence on ASM also suggest an autocrine loop of inflammation whereby ASM-produced TSLP activates the cell in autocrine/paracrine manner and induces inflammatory cytokine (IL-6) and chemokines (IL-8/CXCL8, eotaxin-1/CCL11) release in a MAPK (p38, JNK, Erk) and STAT3-dependent manner. ASM-produced TSLP may also enhance the [Ca $^{2+}$]_i, eventually resulting in increased ASM contractility. Solid line arrows indicate known pathway(s) and dotted line arrows represent plausible pathway(s).

in close vicinity of ASM [35, 55], eosinophils [96] or dendritic cells [44]. Alternatively, the TSLPR activation on ASM by recombinant human TSLP induced a significant release of both eosinophil- (CCL11/eotaxin-1) and neutrophil-attracting (CXCL8/IL-8) chemokines, and pro-inflammatory cytokine IL-6 [86]. Therefore, it is plausible that the ASM-produced TSLP may target the ASM cell itself in an autocrine or paracrine manner (Fig. 2), alike other cytokines and chemokines.

Another group [57] recently confirmed the TSLPR expression on ASM cells. Authors also reported an enhanced expression of both TSLP and TSLPR upon CSE exposure. Interestingly, TSLP stimulation of ASM cells led to enhanced [Ca $^{2+}$]_i responses to bronchoconstrictor agonists, a critical determinant of ASM contractility. Moreover, the use of TSLP and TSLPR blocking antibodies abrogated the CSE-induced [Ca $^{2+}$]_i, suggesting TSLP to be a potential mediator of airway contractility, at least from an ASM cell perspective [57]. Collectively, these studies uncover a novel role of TSLP in modulating the function of ASM that may eventually contribute to airway inflammation and bronchoconstriction in a TSLP/TSLPR-dependent manner. This may also partly explain the attenuated bronchial hyperresponsiveness and inflammation in TSLPR $^{-/-}$ mice [84]. Whether ASM-derived TSLP activates ASM cells in an

autocrine/paracrine manner or is consumed readily and preferably by other cells of local milieu (e.g. mast cells, eosinophils or epithelial cells) (Fig. 2), needs further investigation.

Thymic stromal lymphopoietin in cell migration, survival and proliferation: role in tissue remodelling?

The TSLP was initially identified as a growth factor [33], and induced proliferation in a pro-B cell line Ba/F3. Later, it was shown to stimulate both myeloid and lymphoid cells, notably eliciting the proliferation of naïve T cells [97], pro- and pre-B cells [98]. We have previously reported a critical role of TSLP in wound repair, cell proliferation and migration of airway epithelial cells in allergic asthma [99]. The TSLP also delayed the eosinophil apoptosis, enhanced the intercellular adhesion molecule-1 (ICAM-1), and inflammatory cytokine (IL-6) and chemokine (CXCL8, CXCL1, and CCL2) expression [96].

In context of airways, evolving information suggest the complexity in understanding the exact function of TSLP in tissue remodelling through its preliminary effects on proliferation, migration and survival. Therefore, detailed studies are required to delineate the multiple functions of TSLP (either autocrine or

otherwise) on lung mesenchymal cells which may modulate the airway remodelling, inflammation and bronchoconstriction.

Molecular regulation of thymic stromal lymphopoietin expression

The TSLP expression is under the control of differential regulatory signalling mechanisms, such as p38 and Jun kinase (JNK) in response to respiratory syncytial virus (RSV) infection in airway epithelial cells [100]; cooperation between NF- κ B and interferon regulatory factor-3 (IRF-3) following dsRNA or STAT6 following IL-4 stimulation in NHBE [79]; and IL-1 β - and TNF α -induced NF- κ B activation in NHBE [36]. In ASM, pharmacological inhibitors of MAPK (p38 and ERK1/2) abolished the IL-1 β - and TNF α -induced TSLP production from ASM cells. We recently demonstrated the involvement of NF- κ B, and AP-1-like transcription factors in TNF- and IgE-induced TSLP expression in ASM cells (Fig. 1) [51].

Factors negatively regulating TSLP expression remain poorly described. Interferon-gamma (IFN- γ), transforming growth factor-beta (TGF- β) and IL-17 suppress the release of TSLP by human keratinocytes when stimulated with poly I:C, a TLR3 ligand. However, the factors regulating Th2 inflammation (such as IL-10, TGF- β or IFN- γ) do not show an inhibitory effect on TSLP release from human skin explants stimulated by pro-inflammatory and Th2 cytokines [101]. Nuclear receptors have been suggested to be involved in repressing TSLP expression in both human and murine cells. The GC were shown to repress the TSLP transcription by 'trans-repression' mechanisms mediated by negative GC response elements (nGRE) motifs in GR [102]. The ablation of retinoid X receptors (RXRs) in keratinocytes results in increased TSLP expression in these cells [103]. Moreover, topical application of active vitamin D3 or the low-calcemic analogue MC903 leads to elevated TSLP production from normal keratinocytes [104]. The RXR agonist 9-cis-retinoic acid was shown to inhibit TSLP expression in airway epithelial cells via directly inhibiting NF- κ B [105]; however, the role of NF- κ B remain controversial in this mechanism as another group used more selective synthetic RXR agonists and found opposite results [106]. Interestingly, capsazepine, a widely used transient receptor potential cation channel, subfamily V, member 1 (TRPV1) antagonist was shown recently to completely inhibit the dsRNA-induced epithelial TSLP and TNF- α gene expression [107]. Capsazepine's anti-inflammatory actions were found to be superior over dexamethasone, advocating the exploration of airway pharmacology of novel capsazepine-derived chemical compounds in virus-induced severe asthma.

Thymic stromal lymphopoietin/TSLPR complex signalling

It is surprising that our knowledge of TSLP/TSLPR interaction remains curtailed, despite of a widely accepted critical role of TSLP in driving Th2-type inflammation. The TSLP-mediated signal transduction was earlier shown not to require JAK1 and JAK2, as the dominant negative forms of JAK1 and JAK2 were unable to block TSLP-mediated STAT5 activation [108]. Although multiple studies implicate STAT5 and Tec kinase involvement in TSLPR-signalling [32, 33], no JAK kinases were shown to be involved initially. However, a recent study showed that TSLP requires JAK1 and JAK2 for STAT activation [109]. Authors conclude that the human TSLPR mediates gene regulation not only through STAT5 and STAT3 but also has the potential to mediate transcription via STAT1.

In ASM, TSLP transiently activates all three classes of MAPKs (p38, JNK and Erk1/2), and induces STAT3 but not STAT5 phosphorylation (Fig. 2) [86]. Notably, TSLPR activation in bronchial epithelial cells involves both STAT3 and STAT5 phosphorylation to induce IL-13 production and cell proliferation [99]. It is indeed surprising that TSLP does not induce STAT5 phosphorylation in ASM, particularly since STAT5 is considered to be a signature transcription factor in TSLPR-signalling [33, 99, 108–110]. Although another group [57] later showed STAT5 phosphorylation in chronic (overnight) TSLP stimulation of ASM cells, it might be an indirect effect of ASM-produced mediators instead of TSLP itself. The TSLP was also shown to activate both STAT5 and Akt leading to pro-survival factor Bcl-2 induction in CD8 + T cells, eventually contributing to murine CD8 + T cell homeostasis [97].

Furthermore, a recent study implicates STAT1, -3, -5 and -6 phosphorylation in human primary mDCs, predominantly mediated by robust and sustained JAK1 and -2 activation [111]. In an attempt to define the DC functional plasticity, authors also found that TSLP activates PI-3K/Akt pathway, Erk, JNK MAPK and NF- κ B-dependent pathways in human mDCs [111]. The TSLPR activation on human eosinophils was shown to involve the activation of Erk, p38 MAPK and NF- κ B signalling pathways, but not the STAT5 or STAT3 which are usually activated in other effector cells upon TSLP stimulation [96], partly corroborating the observation of no STAT5 involvement in TSLPR activation in ASM cells.

In summary, TSLPR activation involves components of different signalling pathways, such as protein kinases, MAPKs, JAKs and transcription factors in different cell types. A clear picture of the TSLPR-mediated signalling pathways will be useful for the understanding of the mechanisms by which TSLP exerts its effects, which may eventually lead to potential therapeutic interventions.

Therapies targeting thymic stromal lymphopoietin/TSLPR pathway

Emerging evidence in the pathogenesis of asthma, AD, cancer and COPD has attracted TSLP as a promising therapeutic target. Multiple strategies to design novel TSLP or TSLPR antagonists are in developmental stages. These strategies aimed at the use of TSLP antibodies or antagonists, either alone or in combination with other anti-cytokines, such as IL-4, IL-5, IL-13 and TNF as a treatment of psoriasis, allergic inflammation and fibrotic disorders, summarized in [112]; monoclonal antibodies that target OX40L to inhibit DC-mediated airway inflammation [113]; and targeting the TSLP signalling pathways using a small molecule novel JAK1 and JAK2 inhibitor INCB018424 that was recently shown to inhibit TSLP-induced cutaneous inflammation in its pre-clinical evaluation [114]. Furthermore, a TSLP vaccine is under development for the treatment of Th2-mediated inflammatory diseases, while targeting TSLP or TSLPR in the treatment of tumours is also underway (source: US Patent and Trademark Office). Although various companies are actively engaged in setting pre-clinical evaluation of TSLP/TSLPR antagonists, there is no report of a definitive success of these approaches in clinical trials. As of February 2011, Amgen's AMG 157, a fully human mAb that blocks the interaction of TSLP with TSLPR, is the only antibody that is being investigated in phase I clinical trials for its potential as a treatment for asthma.

Concluding remarks

The TSLP has emerged recently as a key cytokine in initiation and maintenance of Th2-type allergic inflammation. Surprisingly, airway structural cells (i.e. ASM)

have emerged as a rich source of TSLP, with multiple triggers and signalling pathways of its expression (Fig. 1). The β_2 -agonists enhance the cytokine-induced TSLP expression in these cells, an effect that is inhibited when combined with corticosteroids. Despite some important leads, the detailed mechanisms of TSLP expression in ASM remain yet to be established. The TSLP activates a plethora of cells by signalling through TSLPR in a broadly differential manner that involves multiple downstream protein kinases and transcription factors. Further investigation is required to establish specific pathways that can be targeted for therapeutic interventions. In summary, TSLP plays a critical role in initiation and perpetuation of airway inflammation by activating inflammatory [34, 35, 96] and mesenchymal structural cells [86]. Conversely, it may be considered as a homeostatic cytokine that regulates proliferation and tissue repair in airway epithelium through an IL-13-dependent manner [99], and supports early pregnancy by inducing favourable regulatory Th2 immune bias [50]. Therefore, its dual role in disease and homeostasis warrants a note of caution in efforts towards development of TSLP/TSLPR-targeted therapeutic interventions.

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