Thymic stromal lymphopoietin in respiratory disorders: an update on signalling pathway

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Abstract

Introduction
The epithelial cells of airways, skin and gut represent the first barrier of the human body against foreign antigens. The aim of this review is the assessment of thymic stromal lymphopoietin (TSLP) signalling pathway in the genesis of upper airways disorders focusing the attention on sinonasal diseases.

Conclusion
Many experimental data indicate that TSLP plays a critical role in Th2 driven diseases like asthma, atopic dermatitis, allergic rhinitis and chronic rhinosinusitis.

TSLP could be considered in the near future as a therapeutic target for biological therapies of asthma and rhinosinusitis.

Introduction
The epithelial cells (ECs) of airways, skin and gut represent the first barrier of human body against foreign antigens. Several recent studies demonstrated the pivotal role of epithelial derived cytokines in driving the immune response. The aim of this review is the assessment of thymic stromal lymphopoietin (TSLP) signalling pathway in the genesis of upper airways disorders focusing the attention on sinonasal diseases.

We review here TSLP structure, its interaction with the TSLP receptor (TSLP R), its tissue expression and signalling mechanism. Moreover, its actions on priming dendritic cells (DCs) and polarising T cells have also been reviewed, with a focus on the role TSLP plays in chronic inflammatory disorders of the upper respiratory tract.

Discussion

TSLP cytokine
The EC-derived TSLP was first identified in 1994 in supernatants from mouse thymic stromal cell lines ZZ210R.1. Further molecular characterisation in humans showed human TSLP had only 43% amino acids homology with mouse TSLP, with similar biological functions and tissue localization. The human TSLP gene is located on chromosome 5q22 not far from the cluster gene encoding the Th2 cytokines. TSLP is a 140-amino acid IL-7-like 4-helix bundle cytokine mainly expressed by barrier ECs in the thymus, lung, skin, bowel and tonsils and has been recently identified as a key factor in Th2-inflammatory response. TSLP is constitutively expressed in intestinal and thymic ECs. TSLP is involved in tolerance to commensal flora in the bowel and in differentiation of T regulatory cells in the thymus, by modulating the DCs activity. TSLP expression may be induced by different exogenous/endogenous stimuli, as well as pathogens, traumas, infections, allergens, Toll-like receptor ligands, pro-inflammatory and Th2 cytokines.

TSLP R
TSLP R complex is made up of a TSLP-binding chain and the interleukin 7 receptor α (IL7Rα) chain. Human and mouse TSLP R share about 40% of the protein sequence. The human TSLP R gene is located on chromosome Xp22.3 and Yp11.3. TSLP R is expressed on several immune cells: early B and T cell progenitors, peripheral CD4+ T cells, natural killer T cells (NKT), monocytes, mast cells, DCs, as well on heart, skeletal muscle, kidney and liver cells. In humans, TSLP activates the signal transducer and activator of transcription (STAT)-5 and -3 by interacting with the heterodimeric complex

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TSLP R/IL7Rα. It has been shown that TSLP and IL7 receptors use different signal mechanisms to activate STAT5. IL7 activates JAK 1 AND JAK 3, while TSLP was believed not to activate any JAK kinases. In contrast to this view, two recent studies showed activation of JAK1 and JAK2 after TSLP signalling in both human DCs and human CD4+ T cells. JAK 1 and JAK 2 are synergically bound from the IL7Rα and the TSLP R subunits, and, in addition to STAT 5, TSLP stimulation can activate STAT1, STAT3, STAT4, STAT5, and STAT6, as well as JAK1 and JAK2. Presumably, other signalling pathways are activated by TSLP such as PI 3-K/Akt, MAPK and/or Src family Kinase pathways.

TSLP and immune response

The role of TSLP consists mainly in polarising DCs to induce the differentiation of naïve T cells into inflammatory Th2 cells by up-regulating co-stimulatory molecules (CD40, CD80), MHC II molecules and OX40 ligand (OX40L) and MDC (macrophage derived chemokine). TSLP-treated DCs up-regulate Molecules expressed by activated CD4 T cells, 24–48 hours after engagement of T cell receptor (TCR), and not constitutively expressed on naïve T cells. According to some observations, OX40/OX40L interaction is involved in Th2 inflammatory diseases, particularly allergic inflammation, and its role is crucial for T cell activation and polarisation towards a Th2 inflammatory pattern. High levels of OX40L are expressed by TSLP-activated DCs which play an important role in driving Th2 cells expansion by interacting with OX40+ CD4 T cells.

TSLP and adaptive immunity

TSLP acts on CD4+ T cells, not only indirectly through DCs and OX40L, but also in a direct way. In vitro studies demonstrated that TCR-engagement and consequent activation of T cells induce TSLP R expression and proliferation of pro-activated CD4+ T lymphocytes leading to their responsiveness to TSLP stimulation. T regulatory cells (CD4+/CD25+/Foxp3+) are a small population of DCs with a suppressive and regulatory function, which are thought to be important in the maintenance of tolerance towards self antigens. Their differentiation is under the control of the transcription factor forkhead box protein 3 (Foxp3). In thymic medulla, approximately 50% of CD4+/CD8−/CD25-lymphocytes bearing TCR with high affinity for self peptide-MHC complexes are induced by Hassall’s corpuscles ECs TSLP to differentiate into Treg cells in a DC-dependent manner.

TSLP action is also explicated on activation and differentiation of CD8+ T cells into pro-allergic cytotoxic T cells that produce large amounts of IFN-γ. Similarly to CD4+ T cells, TSLP can induce TSLP R expression on TCR activated CD8+ T cells increasing their survival through Bcl-2 anti-apoptotic protein.

The role of TSLP on B cells development is still unclear: in vitro studies suggested that TSLP can contribute to B lymphopoiesis, being active on pro-B cells derived from foetal liver. In vivo results are contradictory about the role of TSLP in B cells development, as it has been demonstrated by Carpino et al. that B cells, as well as T cells, development is normal in TSLP R−/− mice. This discrepancy can be the result of different levels of circulating TSLP in transgenic compared to wild mice.

TSLP and innate immunity

TSLP can also influence cells of innate immunity like mast cells, basophils, eosinophils and NKT cells. NKT cells, like the other T cells, develop from thymocytes progenitors and after TCR activation can rapidly produce IL-4 and IFN-γ. High percentages of NKT cells have been found in broncho-alveolar lavage fluid in asthmatic patients with AHR. It has been observed that TSLP, in these patients, may induce NKT cells to produce IL-13, which, in turn, is able to increase airway inflammation and reactivity.

Mast cells, basophils and eosinophils are cells that commonly contribute to immediate hypersensitivity reactions in allergic responses.

Mast cells are one of the identified non-epithelial sources of TSLP. Following IgE receptor activation, mast cells are able to produce TSLP and up-regulate the expression of TSLP R complex, leading to TSLP response in a pro-inflammatory environment (IL1, TNF alpha). TSLP stimulation of mast cells do not

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induce their degranulation, but it promotes the release of several Th2 cytokines (IL5, IL6, IL13, TNF alpha, GM-CSF) and chemokines (CCL1 and CCL8) 31.

The pivotal role of mast cells in the induction of TSLP production has been demonstrated by Miyata et al. 32 In an allergic rhinitis (AR) model of mast cell deficient mice where TSLP expression was abolished.

Moreover, TSLP is able to activate common progenitors of basophils and eosinophils. Basophils represent less than the 1% of circulating leukocytes and they have been identified as a potential source of TSLP, so contributing together with the other epithelial-derived cytokines to the Th2 allergic response 31,33. Eosinophils represent less than the 1% of circulating leukocytes and eosinophils. Basophils represent less than the 1% of circulating leukocytes and they have been identified as a potential source of TSLP, so contributing together with the other epithelial-derived cytokines to the Th2 allergic response 31,33. Eosinophils represent less than the 1% of circulating leukocytes and they have been identified as a potential source of TSLP, so contributing together with the other epithelial-derived cytokines to the Th2 allergic response 31,33,34.

Human eosinophils express the functional TSLP R complex and can be directly activated by TSLP in a dose-dependent and specific manner. Many effects of TSLP on eosinophils have been reported: delayed apoptosis, up-regulation of cell surface expression of adhesion molecule CD18 and intercellular adhesion molecule-1, with down-regulation of L-selectin, enhanced adhesion to fibronectin, and augmented release of inflammatory cytokine IL-6 and chemokines CXCL8, CXCL1 and CCL2 31,35. TSLP has been shown to regulate the above reported effects on eosinophils through the activation of extracellular signal-regulated protein kinase, p38 mitogen-activated protein kinase, and NF-kB signalling pathway, and not through STAT-5 and STAT-3, which are usually activated by TSLP stimulation of other effector cells 35.

Moreover, group 2 innate lymphoid cells (ILC2s) have been recently shown to play a critical role in the early phases of the development of allergic inflammation at multiple barrier surfaces. The EC-derived cytokines IL-25, IL-33 and TSLP regulate the activation and effector functions of ILC2s 36.

TSLP and ECs-derived cytokines network
Several recent studies investigated the role of ECs in licensing the functions of specific immune cell populations in the airway and gastrointestinal tract. It has been demonstrated that ECs are not only a physical barrier in the mucosal sites, but also play a key role in the initiation, regulation and resolution of innate and adaptive immune responses. TSLP and other epithelial-derived cytokines (IL25 and IL33) constitute a wide and complex network for the regulation of immunity, inflammation and immune regulation. This triad of cytokines share a common cellular source (ECs) but they differ in terms of structure and cell population targets. Tissue cytokines promote Th2 response in vivo and are important in modulating mucosal immune response 37.

TSLP, IL25 and IL33 are able, directly and/or indirectly, to initiate the inflammatory cascade, its progression and regulation, and they seem to be involved in several diseases 31,38-43. Interactions among tissue cytokines are regular and not entirely known. IL25 and IL33 together are able to induce TSLP mRNA in ECs 34,45. These cytokines can up-regulate with a positive enhancing loop with their cognate receptors 46,47 and IL33 is able to sensitize mast cells to TSLP 48.

Both TSLP and IL25 have many effects on APCs 39,49 and CD4+ naïve T cells, the first one by up-regulating GATA3 expression and IL4 production 50, the second one by up-regulating IL4, IL5 and IL13 production and activating T CD4+ memory cells 39,45. TSLP, in a pro-inflammatory environment (with or without IL33), promotes mast cells production of IL4, IL5 and IL13 and Th2-like chemokines 51,48. IL33 can furthermore increase mast cells survival and sensitize them to TSLP stimulation 49. Basophils are induced to produce cytokines by IL33 43 and IL25 can increase eosinophils survival 31,51.

All these data indicate that ECs may have a critical role in the initiation of Th2 immune responses at mucosal sites through TSLP, IL25 and IL33 production.

TSLP and diseases
Many experimental data indicate that TSLP plays a critical role in Th2-driven response. Its increased production has been shown in target tissues of patients with Th2-related diseases like atopic dermatitis (AD), asthma and AR.

Recently, TSLP has been found highly expressed in sinus mucosa of patients with chronic rhinosinusitis with nasal polypos (CRSwNP) and its role in cancer escape has been recently studied in patients with breast and pancreatic tumors 52-56.

TSLP and AD
AD is a chronic inflammatory skin disorder of variable severity characterised by eczematous skin lesions associated with itching. It has a strong genetic component and may affect children (10%–20%) and adults (1%–3%). AD is characterised by proliferation of epidermal keratinocytes and abnormal keratinisation; skin lesions contain immune infiltrates of Th2 cells and DCs. High levels of total IgE and eosinophil numbers in the peripheral circulation are common in these patients 56.

High levels of TSLP and inflammatory cytokines like IL-1b, TNF-alpha, IL-4, and IL-13 have been shown in keratinocytes from skin biopsy specimens of patients with AD 32, suggesting a feed-forward inflammatory cascade that synergise to induce TSLP expression by keratinocytes 36,39. Suggesting that TSLP may play a role in...
the Th2 inflammatory aspect of this disease.

In murine models of AD, TSLP R (-/-) mice showed a significant decrease of skin infiltrating eosinophils and tissue Th2 cytokines concentration, while intradermal injection of anti-TSLP antibodies were able to block the development of allergic skin inflammation. TSLP has also been implicated in the phenomenon referred to as the atopic march, that refer to the chance of subjects with AD to develop AR and asthma in the future. Several murine models of induced TSLP expression in keratinocytes result in allergic airway inflammation after intranasal challenge, suggesting that TSLP might be an important factor contributing to this progression from AD to AR and asthma.

**TSLP and asthma**

Asthma is a disease characterised by chronic airway inflammation that is mediated by Th2 cells and their related cytokines, IgE production, innate immune cells recruitment and mucus production. The inflammatory mediators released by activated mast cells in a IgE-dependent way are responsible of an increased inflammatory cell infiltrate and mucus production, bronchial smooth muscle contraction and increased vascular permeability.

Several recent studies suggest TSLP may play a key role in the pathogenesis of allergic and non-allergic asthma, both phenotypes being often characterised by tissue eosinophilia and Th2 cytokines. High levels of TSLP have been observed in bronchial ECs derived from asthmatic patients, where the levels of TSLP have been found to be inversely related to lung function. Zhang et al., by blocking TSLP signalling through the soluble TSLP R-Ig in a murine asthma model, were able to prevent airway inflammation, by down-regulating co-stimulatory molecule expression (CD40, CD80 and CD86) on pulmonary DCs.

Moreover, Semlali et al. demonstrated that TSLP is able to promote epithelial airway proliferation and epithelial injury repair trough IL13 production. ECs from asthmatic patients increased TSLP mRNA levels after IL13 stimulation and such cells, after TSLP stimulation, were able to increase IL13 production in a dose-dependent manner. These findings suggest a positive enhancing loop between TSLP and IL13 in asthmatic patients.

The above data underline that TSLP plays an important role in promoting aberrant Th2 response to antigens.

**TSLP and AR**

The prevalence of AR increased remarkably in the last years with high social costs. In AR patients, nasal mucosa, after exposure to allergens, is involved by a chronic inflammatory process with increased serum IgE, tissue infiltration of eosinophils and Th2 cells and mucus hyperproduction, resulting in a Th1 and Th2 cells imbalance with hyperproduction of IL4, IL5 and IL13. Xu et al. demonstrated that TSLP and IL25 were up-regulated in the nasal lavages and in nasal ECs stimulated with Poly I:C; moreover, they showed a positive correlation between ECs production of TSLP and IL25, confirming that the epithelial layer is not only a mechanical barrier but a key factor in the initiation and maintenance of the inflammatory environment. Several authors also showed a positive correlation between TSLP and IL4 level, strictly linked with AR severity. The positive correlations observed between TSLP and Th2 cytokines (IL4) and between TSLP and epithelial derived cytokines (IL25) emphasises the central role of TSLP in AR pathogenesis.

**TSLP and CRS**

CRS is a common disease that can significantly decrease quality of life and require significant direct medical expense. The relative roles of initiating events, environmental factors and host susceptibility factors are currently unknown. CRS is a multifactorial disease, where contributing factors may be mucociliary impairment, bacterial infection, allergy, swelling of the mucosa or, rarely, physical obstruction caused by anatomical variations in the nasal cavities or paranasal sinuses. CRS is commonly classified into two phenotypes, CRSwNP and without nasal polyps (CRSsNP). The reason why polyps develop in some patients and not in others remains unknown. NPs consist of connective tissue, oedema and inflammatory cells like eosinophils (the most common), neutrophils, mast cells, plasma cells, lymphocytes and monocytes. Nasal polyps (CRSsNP) have been associated with high levels of IgE, local nasal IgE production, sinus inflammatory infiltrate rich in eosinophils, eotaxin and eosinophil cationic protein (Th2 driven disease). CRSsNP shows a less defined Th2 profile and sinus inflammatory infiltrate rich in neutrophils, as well IFNγ and TGFβ production have been reported.

In a recent report, Liu et al. investigated the role of TSLP in NP development. These authors demonstrated that TSLP levels in the nasal epithelial layer were higher in patients with NPs compared to patients without NPs and their data could not be explained by atopy. In the same report, authors observed that DCs, obtained from NP mucosa stimulated with NP proteins extract, showed higher expression of TSLP R
**Figure 1:** CRS pathogenesis pathway. After various triggers, epithelial cells start to produce TSLP. In the early phases of the disease, TSLP binds its receptor TSLP R on cells of the innate immunity (eosinophils, mast cells) and of the adaptive immunity (dendritic cells and T CD4 cells) in the same way on CRSwNP and CRSsNP, starting a common inflammatory pathway. The second event that happen that probably play a central role on differentiating the two disease subtypes is the engagement of the OX40 on the T CD4 cells with OX40L on dendritic cells, with this link, CRSwNP is polarised towards a Th2 disease, with eosinophils infiltration, Th2 cytokines production and local nasal IgE presence, on the other hand, CRSsNP, that is not able to create the cross-talk between dendritic cells and T CD4 cells trough OX40L/OX40, will be polarised trough a Th1 inflammation pattern, characterised by fibrosis, Th1 cytokines, increased TGFβ production and Treg cells. CRSwNP, chronic rhinosinusitis with nasal polyps; CRSsNP, chronic rhinosinusitis without nasal polyps; TSLP, thymic stromal lymphopoietin; ECP, eosinophil cationic protein; IL, interleukin; TGF, transforming growth factor; TNF, tumour necrosis factor; Th, T-helper cell; Treg, T-regulatory cell.

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and were able to up-regulate OX40L compared to DCs derived from controls. These data indicate that the expression of TSLP R make many cells, apart from DCs, responsive to TSLP produced by nasal ECs, promoting a Th2-inflammatory response.

Kimura et al. demonstrated that immunoreactivity for TSLP was higher in nasal mucosa of AR and in NPs from allergic patients compared to non-allergic subjects. Moreover, eosinophils count and IgE levels in NPs positively correlate with TSLP expression in several cell types (epithelial, endothelial, fibroblast and inflammatory cells).

Boita et al. recently demonstrated a statistically significant difference in TSLP R expression in CRSwNP and CRSsNP compared to normal subjects, both in the inflammatory and ECs, with no significant difference between CRS subtypes (CRSwNP and CRSsNP). The cells with the highest TSLP R immunostaining score were eosinophils, mast cells and histiocytes in agreement with the data reported by Alahkverdi and colleagues who showed that eosinophils and mast cells express the functional TSLP R complex and are able to respond to TSLP signals in the presence of inflammatory environment. Moreover, we observed that the number of OX40L positive cells, as well as mRNA expression of OX40L, are increased in the lamina propria of sinus tissue of patients with CRSwNP with a significant correlation with the eosinophilic infiltration compared to CRSsNP (submitted data). This observation suggests an early common pathway which starts from the epithelial barrier through TSLP-TSLP R as a possible driving factor in the pathogenesis of CRS. The axis TSLP-OX40 should play a major role in promoting Th2 polarisation and eosinophilic inflammation only in CRSwNP (submitted data) (Figure 1).

Many data suggest that TSLP play a central role in NPs development. Increased TSLP levels have been reported in NP and TSLP drive DCs towards a Th2 phenotype by up-regulating OX40L on DCs surface. Moreover, TSLP levels positively correlate with eosinophil tissue infiltrate and IgE levels in allergic subjects with NP. These findings suggest that TSLP-TSLP R interaction at the level of epithelial layer may play an important role in the pathogenesis of CRS characterised by Th2 inflammatory changes of sinus mucosa. The emerging concept of tissue-specific control of immunity, with TSLP secretion by ECs acting as an initial factor in the CRS cascade, can partially explain the early stages of the disease, while the mechanisms involved into the NPs formation are still unknown.

Conclusion

Asthma is a common comorbidity in subjects with AR and CRS and epidemiological surveys have suggested a close connection between upper and lower airway diseases expressed as the 'united airways concept'.

The recent findings of TSLP involvement in both upper and lower airway diseases suggest that this cytokine may partially explain the similar Th2 inflammatory pattern. According to a recent study by Zhang et al. where TSLP signalling was successfully blocked by a soluble TSLP R-Ig in a murine asthma model, TSLP could be considered in the near future as a therapeutic target for biological therapies of asthma and rhinosinusitis.

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