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REVIEW



## Thymic stromal lymphopoietin: its role and potential as a therapeutic target in asthma

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### ABSTRACT

**Introduction:** Thymic stromal lymphopoietin (TSLP), an epithelial cytokine (alarmin), is a central regulator of the immune response to inhaled environmental insults such as allergens, viruses and pollutants, initiating a cascade of downstream inflammation. There is compelling evidence that TSLP plays a major role in the pathology of asthma, and therapies that aim to block its activity are in development.

**Areas covered:** We review studies conducted in humans and human cells, largely published in PubMed January 2010–October 2019, that investigated the innate and adaptive immune mechanisms of TSLP in asthma relevant to type 2-driven (eosinophilic/allergic) inflammation and non-type 2-driven (non-eosinophilic/non-allergic) inflammation, and the role of TSLP as a mediator between immune cells and structural cells in the airway. Clinical data from studies evaluating TSLP blockade are also discussed.

**Expert opinion:** The position of TSLP at the top of the inflammatory cascade makes it a promising therapeutic target in asthma. Systemic anti-TSLP monoclonal antibody therapy with tezepelumab has yielded positive results in clinical trials to date, reducing exacerbations and biomarkers of inflammation in patients across the spectrum of inflammatory endotypes. Inhaled anti-TSLP is an alternative route currently under evaluation. The long-term safety and efficacy of TSLP blockade need to be evaluated.

### ARTICLE HISTORY

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### KEYWORDS

Alarmin; AMG 157; airway; CSJ117; epithelium; GSK2618960; inflammation; tezepelumab; TSLP; type 2

## 1. Introduction

Asthma is a common lower respiratory disease, generally characterized by chronic inflammation of the airways. The hallmarks of asthma include variable expiratory airflow limitation and variable symptomatology, both of which are commonly triggered by various epithelial insults such as viruses, allergens, bacteria, air pollutants and other environmental irritants. With prolonged disease, airway limitation may become persistent. Most patients with asthma have mild disease, but approximately 5–10% of patients have severe disease that requires high-dosage inhaled corticosteroids and additional medications to achieve disease control [1,2]. Furthermore, many patients with severe asthma can have disease that remains uncontrolled despite such therapy [1–3].

The inflammation associated with asthma is heterogenous and has been associated with multiple inflammatory endotypes. The most common endotypes include allergic and eosinophilic inflammation, collectively referred to as type 2 (T2) disease. Population-based studies have consistently concluded that the majority of patients with asthma have T2-driven inflammation [4–7]. Patients who are characterized as having either very low or absent signs of T2 inflammation may instead have neutrophilic or paucigranulocytic inflammation, generally in the context of inhaled corticosteroid therapy [8–10]. Within any individual patient with asthma, there may be evidence of multiple upregulated inflammatory pathways and

it can be difficult to identify a single predominant endotype, which further limits the predictive value of currently available biomarkers. For patients with severe asthma whose disease is not adequately controlled by inhaled therapies, an understanding of the patient's inflammatory endotype(s) helps inform selection of the optimal add-on treatment, including targeted biologic therapies [1,2].

In recent years, the downstream effectors of allergic and eosinophilic inflammation have been the focus of severe asthma research and treatment. These have included immunoglobulin (Ig) E, sputum and blood eosinophils, interleukin (IL)-4, IL-5 and IL-13. This research has resulted in five approved biologic therapies for patients with moderate-to-severe allergic and/or eosinophilic asthma, and all have demonstrated higher efficacy in patients with eosinophilic inflammation than in those without eosinophilic inflammation [11–15]. There are currently no approved biologic treatment options for patients with moderate-to-severe asthma that is characterized by non-eosinophilic inflammation.

The immunology of thymic stromal lymphopoietin (TSLP), an epithelial cytokine, provides an opportunity for a novel approach to treat asthma inflammation. A member of a class of epithelial cytokines commonly referred to as alarmins (whose other members are IL-25 and IL-33), TSLP is released by airway epithelial cells in response to various environmental insults, including viruses, bacteria, allergens, chemical irritants and physical injury [16,17]. Functionally, TSLP is a key

**Article highlights**

- There is a critical need for new therapies to treat patients with severe, uncontrolled asthma, which can be difficult to treat owing to the heterogeneity of airway inflammation.
- Thymic stromal lymphopoietin (TSLP) is a cytokine primarily expressed by the airway epithelium and released in response to environmental insults, instigating a range of downstream inflammatory processes.
- TSLP expression is increased in the airways of patients with asthma compared with healthy individuals, correlating with disease severity and lung function; polymorphisms in the *TSLP* gene are associated with asthma.
- Evidence indicates that TSLP is a key mediator of asthma pathophysiology, driving eosinophilic (allergic and non-allergic) inflammation, non-eosinophilic inflammation and structural changes to the airway, through its actions on a wide variety of adaptive and innate immune cells and structural cells.
- Clinical trials of TSLP blockade, delivered via a systemic route, have produced positive results in a broad population of patients with asthma, reducing exacerbations and multiple biomarkers of inflammation while improving lung function.

This box summarizes key points contained in the article.

instigator of the immune response to environmental insults, initiating a range of downstream inflammatory pathways. While TSLP drives a pronounced T2 inflammatory response [18–20], there is emerging evidence of TSLP involvement in non-T2 processes involving interactions with both immune and structural cell types. The considerable scope of effects mediated by TSLP is illustrated by the wide range of cell types that express the TSLP receptor (TSLPR), including hematopoietic progenitor cells, eosinophils, basophils, mast cells, airway smooth muscle cells (ASMCs), group 2 innate lymphoid cells (ILC2s), lymphocytes, dendritic cells and monocytes/macrophages [21,22]. In addition to its actions on specific cell populations, the possibility that TSLP serves as a key mediator between immune cell types and structural cells in the airway milieu is intriguing and is an area of ongoing research.

In individuals with asthma, as well as those with other inflammatory diseases such as atopic dermatitis, TSLP production appears to be dysregulated. Several studies have shown that TSLP expression is elevated in patients with asthma compared with healthy individuals in inner and outer epithelial layers of airway biopsies [23–30] and in samples of serum [31,32], sputum [33], exhaled breath condensate [34] and bronchoalveolar lavage fluid [30,35]. Furthermore, the level of TSLP expression in patients with asthma has been shown to correlate with airway obstruction and disease severity [25,29,33,35,36]. Various elements of asthma pathophysiology, including airway hyperresponsiveness, mucus overproduction and airway remodeling, are believed to be at least partly driven by TSLP via its downstream, pro-inflammatory effects involving cytokines such as IL-4, IL-5 and IL-13 [37]. The role of TSLP in asthma is underscored by genome-wide association studies that have identified associations between asthma risk and single-nucleotide polymorphisms (SNPs) in the *TSLP* gene [38–40]. These include rs1837253 [41,42], which has been shown to regulate TSLP production in nasal epithelial cells

[43] and influence asthma manifestation [44]. TSLP has also been implicated in aspirin-exacerbated respiratory disease (AERD), which is characterized by asthma, chronic rhinosinusitis with nasal polyps, and intolerance of cyclooxygenase-1 inhibitors. Examination of nasal polyp tissue from individuals with AERD and those with chronic rhinosinusitis without AERD demonstrated that TSLP mRNA expression was increased with AERD and with markers of mast cell activation and prostaglandin D2 expression [45].

The compelling evidence of TSLP's role in the pathogenesis and pathology of asthma has led to the development of anti-TSLP monoclonal antibodies as a potential therapeutic option for these patients. The results of clinical studies of anti-TSLP therapy [46,47] have provided the strongest evidence to date for a major role for TSLP in asthma. The purpose of this review is to summarize the available data regarding the mechanisms of action of TSLP in human asthma across the spectrum of inflammatory endotypes, with the goal of elucidating the therapeutic potential of novel therapies that block TSLP activity. Although the biology of the TSLP pathway appears to be similar in humans and rodents, the ability to use rodent models to study the impact of blocking TSLP in humans is limited by the generally low translatability of rodent models to complex heterogeneous human disease [48,49]. As such, we have excluded studies of TSLP in animal models of asthma from this review and, instead, have emphasized observations with direct clinical relevance.

To inform the review, we conducted a literature search of the PubMed database for articles in English published between 1 January 2010 and 1 October 2019 using the search terms (TSLP[title/abstract] OR thymic stromal lymphopoietin [title/abstract]) AND asthma\*[title/abstract], employing the 'Humans' species filter and excluding review articles. The results from this search were screened for relevance, i.e. whether they contained information about sites of TSLP expression, TSLP effector cells, or physiological or clinical effects of TSLP, and were supplemented by further relevant articles known to the authors. The included articles are summarized in Table 1.

## 2. Innate immune mechanisms of action of TSLP relevant to T2-driven (eosinophilic/allergic) inflammation in asthma

Several local effector cells play a role in propagating T2 inflammatory responses, and the interaction between the airway epithelium and these cells is an important process driving eosinophilic inflammation. Evidence that TSLP directly activates innate immune cells involved in T2 inflammatory processes in human asthma is discussed in this section, with evidence for the role of TSLP in adaptive immune cell-mediated inflammatory processes discussed in section 3. This evidence is summarized in Figures 1, 2 and 3.

### 2.1. TSLP and group 2 innate lymphoid cells (ILC2s)

ILC2s are lineage-negative cells, lacking antigen-recognition receptors, which provide the primary early innate cellular source of T2

**Table 1.** Studies of TSLP with relevance to asthma, relating to cellular sites of expression/localization, effector cells and physiological and clinical effects.

	Studies in patients or patient samples*		Studies in samples from healthy individuals or in human cell lines		Total studies
	Number of studies	References	Number of studies	References	
<b>Cellular sites of TSLP expression/localization‡</b>					
Airway epithelial cells§	14	[25,26,29,30,33,50–54,71,116,122,127]	10	[18,19,55–59,90,123,125]	24
ASMCs	1	[24]	5	[19,93,119,123,124]	6
Bone marrow mesenchymal stromal cells	0	-	1	[83]	1
Bronchial endothelial cells	4	[25,29,30,116]	0	-	4
Dendritic cells	0	-	2	[100,101]	2
Fibroblasts	1	[116]	3	[19,123,127]	4
Monocytes/macrophages	3	[25,29,30]	1	[100]	4
Mast cells	6	[24,25,29,30,91,116]	2	[19,92]	8
Neutrophils	3	[29,30,116]	0	-	3
<b>TSLP effector cells</b>					
Airway smooth muscle cells	2	[24,122]	5	[60,93,119–121]	7
Basophils	6	[74,85–89]	2	[73,84]	8
Bronchial epithelial cells	1	[122]	1	[61]	2
Dendritic cells	5	[26,85,104,105,114]	9	[19,95–98,102,103,107,113]	14
Eosinophils	5	[28,36,46,81,82]	1	[80]	6
Fibroblasts	0	-	3	[125,126,128]	3
Hematopoietic progenitor cells	3	[74–76]	1	[73]	4
ILC2s	5	[27,65,67,71,72]	1	[66]	6
Mast cells	1	[24]	3	[18,90,93]	4
Monocytes/macrophages	0	-	2	[94,95]	2
Neutrophils	0	-	1	[115]	1
T cells, CD4 <sup>+</sup>	3	[74,104,109]	6	[19,95,98,102,108,113]	9
T cells, CD8 <sup>+</sup>	0	-	1	[110]	1
T cells, regulatory	2	[32,106]	0	-	2
Th2 cells	4	[104,105,109,114]	6	[97,98,102,103,107,113]	10
Th17 cells	1	[114]	1	[113]	2
<b>Physiological and clinical effects of TSLP</b>					
Airway inflammation¶	7	[25,28,35,36,46,47,134]	N/A	N/A	7
Airway obstruction/reduced lung function	7	[25,29,33,35,36,46,47]	N/A	N/A	7
Airway remodeling	3	[50,129,135]	4	[121,125,126,128]	7
Epithelial barrier maintenance	1	[50]	1	[61]	2

\*Patients with asthma (studies may also include healthy controls or patients with other atopic diseases), except for two studies in patients with atopic dermatitis [85,114].

‡Cellular sites of TSLP mRNA expression and/or TSLP protein expression/localization.

§Includes bronchial, nasal and unspecified airway epithelial cells. Owing to the very large number of studies indicating that TSLP is expressed in airway epithelial cells, not all have been cited in the main text.

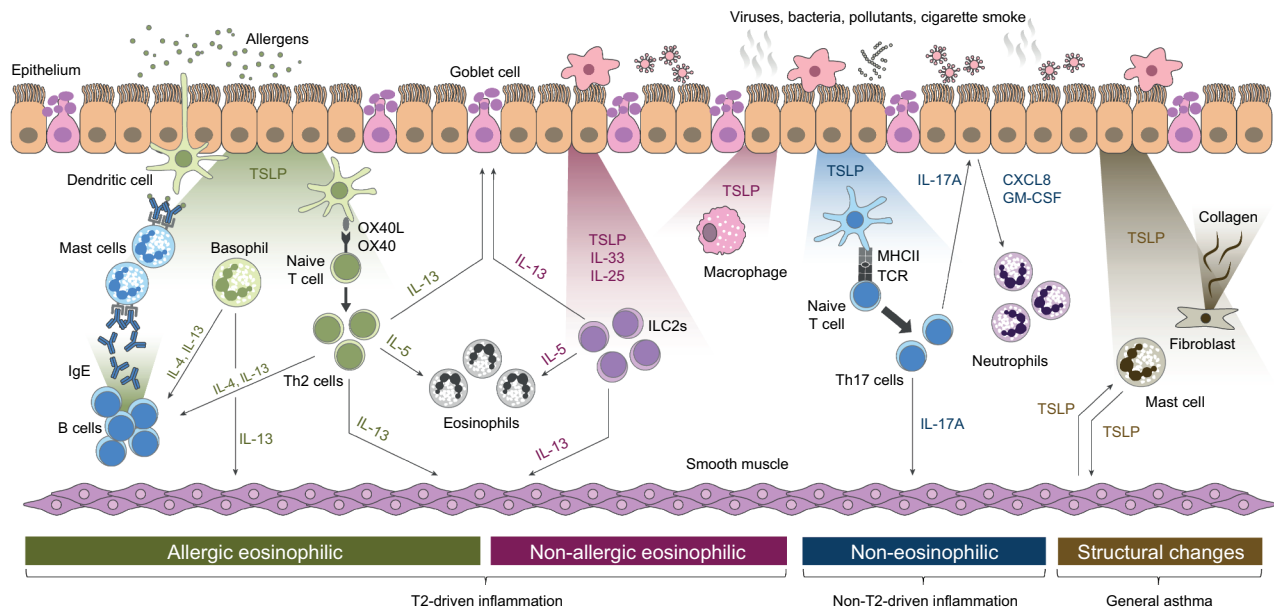
¶Defined as effects on eosinophil and/or neutrophil numbers in patient sputum, bronchoalveolar lavage fluid or lung tissue biopsies.

ASMC, airway smooth muscle cell; ILC2, group 2 innate lymphoid cell; N/A, not applicable; Th, T helper; TSLP, thymic stromal lymphopoietin.

cytokines that drive eosinophilic inflammation. ILC2s produce substantial amounts of T2 cytokines including IL-5, IL-13 and IL-9 following activation by alarmin cytokines such as TSLP, IL-25 and IL-33 [63–65]. This effect is enhanced in the presence of IL-2 and IL-7 [66]. TSLP can synergize with IL-25 or IL-33 to promote ILC2 production of IL-5 and IL-13 [65] and prolonged ILC2 survival [66]. Activation of ILC2s by IL-33 and TSLP results in upregulation of surface expression of c-Kit and downregulation of IL-7R $\alpha$  and CRTH2, suggesting that alarmin cytokines can create heterogeneous populations of ILC2s [66]. The functions of the various populations remain to be clarified.

With respect to human asthma models, Chen *et al.* (2017) reported that, in mild asthmatics, there was a rapid and significant increase in sputum ILC2s expressing high levels of IL-5 and IL-13 within 24 hours post-allergen inhalation challenge [67]. Phenotypic analysis of ILC2s in this study showed upregulation of TSLPR on ST2<sup>+</sup>ILC2s, indicating that increased responsiveness of ILC2s to TSLP within the airways may help to propagate eosinophilic inflammation. Other studies have shown that ILC2

numbers are increased in patients with severe asthma and persistent eosinophilia compared with those with mild asthma, with the greatest number of airway IL-5<sup>+</sup>IL-13<sup>+</sup>ILC2s observed in patients with uncontrolled eosinophilia despite treatment with high-dose oral corticosteroids [68–70]. In endobronchial biopsies from prednisone-dependent patients with severe asthma, ILC2s were found to co-localize to TSLP-immunopositive regions [25]. Similarly, the number of ILC2s in nasal biopsies have been found to correlate positively with nasal tissue TSLP levels in patients with severe asthma and chronic rhinosinusitis [27]. Liu *et al.* (2018) reported that dexamethasone treatment following *in vitro* stimulation of peripheral blood cultures from patients with severe asthma using *Aspergillus* or IL-2/IL-33 resulted in inhibition of IL-5 production by ILC2s [71]. In contrast, dexamethasone had no effect on ILC2s from the airways, indicating compartmental differences in steroid resistance in ILC2s [71]. This was attributed to higher levels of TSLP in the airways. Specifically, the study showed that the inhibitory effects of dexamethasone on airway ILC2s was reduced in the presence of TSLP and IL-7,



**Figure 1.** The role of TSLP in driving disease mechanisms in different asthma endotypes. In allergic eosinophilic inflammation, TSLP initiates pathways involving Th2 lymphocytes, basophils and mast cells to drive airway eosinophilia. In non-allergic eosinophilic inflammation, TSLP activates innate lymphocytes such as ILC2s that contribute to airway eosinophilia. The mechanisms underlying non-eosinophilic inflammation require further elucidation, but TSLP-related processes involving Th17 lymphocytes and neutrophils appear to be involved. TSLP also mediates structural mechanisms that contribute to airway remodeling, involving airway smooth muscle cells and fibroblasts. Further details of the mechanisms are provided in Figures 2–5. Figure adapted, with permission, from Brusselle G & Bracke K, *Ann Am Thorac Soc.* 2014;11 Suppl 5:S322–8 [62]. CXCL8, chemokine (C-X-C motif) ligand 8; GM-CSF, granulocyte-macrophage colony-stimulating factor; IgE, immunoglobulin E; IL, interleukin; ILC2, group 2 innate lymphoid cell; OX40 L, OX40 ligand; T2, type 2; Th, T helper; TSLP, thymic stromal lymphopoietin.

and this was found to be dependent on MEK and STAT5 signaling [71]. Three genes, *CBX7*, *MEK2* and *TRL2*, have been identified in TSLP-stimulated lymphoid cells that are resistant to dexamethasone treatment [72]. TSLP itself can induce expression of MEK2, which translocates to the nucleus and interacts with CBX7, suggesting a positive feedback regulatory pathway [71]. It is proposed that, while dexamethasone may attenuate the pro-inflammatory activity of ILC2s driven by IL-33, TSLP may have a role in conferring steroid resistance of ILC2s.

## 2.2. TSLP and hematopoietic progenitor cells

There is evidence to support an association between allergen-induced asthmatic responses and mobilization of eosinophil progenitor cells (EoPs) from the bone marrow. Affected tissues support local differentiation, proliferation, maturation and activation of EoPs that home to the site of allergen exposure in airway disease. TSLP has been shown to drive activation, migration and local differentiation of EoPs within the airways.

Cord-derived hematopoietic progenitor cells cultured overnight with TSLP at nanomolar levels upregulate IL-5R $\alpha$  expression and then stimulate significant outgrowth of eosinophil/basophil colony-forming units (Eo/Bo-CFUs) in combination with IL-3 or GM-CSF [73]. In addition, increased eosinophilopoietic activity was evident in bronchial epithelial supernatants from patients with severe eosinophilic asthma compared with mild asthmatic and healthy controls. This activity was attenuated by a receptor-blocking antibody to TSLP [74]. At picogram levels, TSLP stimulated the outgrowth of Eo/Bo-CFUs, with additive effects in the presence

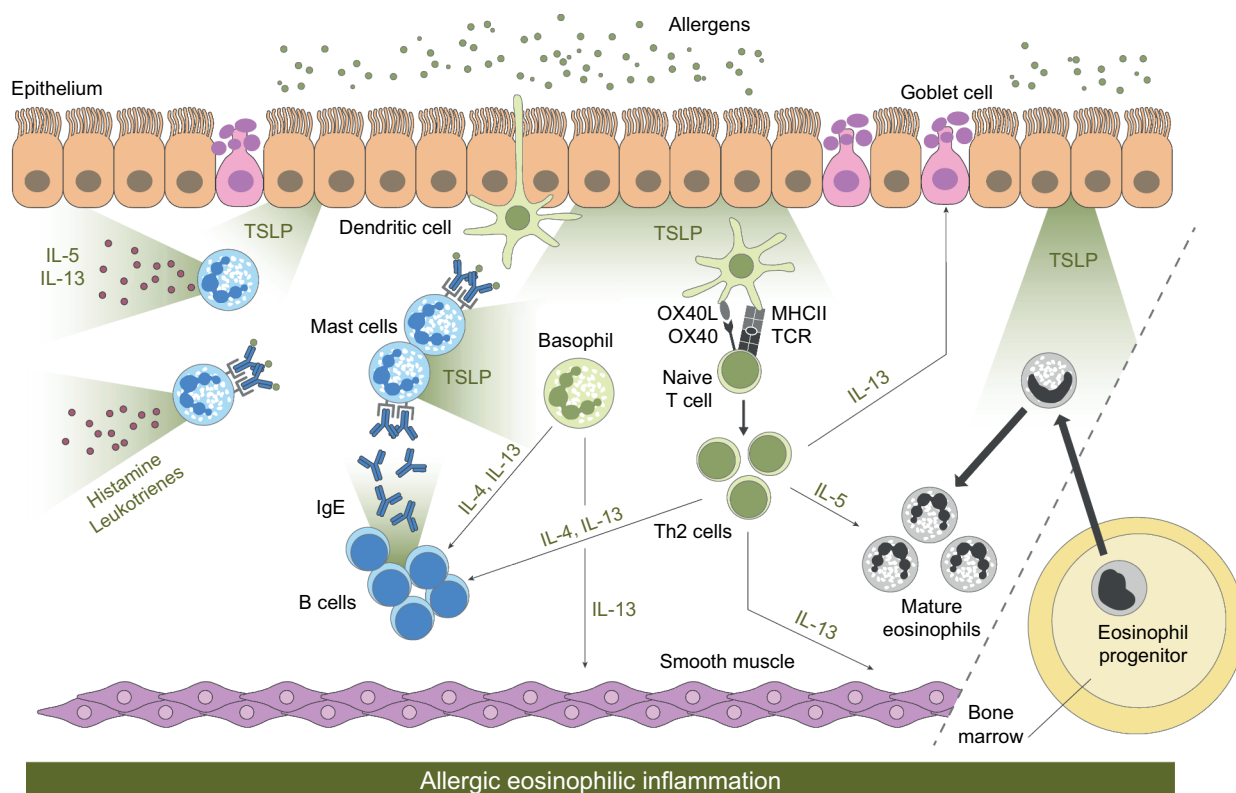
of IL-5 [74]. At the mRNA level, a synergistic increase of GATA-2 and CEBP $\alpha$  in CD34 $^{+}$  cells was observed in the presence of TSLP and IL-5 [74]. Collectively, these findings indicated that eosinophilopoiesis is not solely driven by IL-5, but rather is a complex process involving the interaction between local and systemically elaborated growth factors, including TSLP.

Migration of precursor cells to the airways is an important component of driving local eosinophilic inflammation. Pre-exposure to TSLP and IL-33 primes migration of progenitor cells towards the chemoattractant SDF-1 $\alpha$  (CXCL12) [75]. This implies that the airway epithelium can locally release alarmin cytokines that enhance the migrational responsiveness of CD34 $^{+}$  progenitor cells. In addition, CD34 $^{+}$  primitive progenitor cells express TSLPR and overnight stimulation with TSLP results in a dose-dependent release of IL-5, IL-13, GM-CSF and chemokines including CCL22, CXCL8 and CCL1 [75,76]. This indicates that TSLP not only drives local maturation of eosinophil-lineage committed progenitor cells but may also promote pro-inflammatory function and migration of primitive progenitor cells.

## 2.3. TSLP and eosinophils

Eosinophilic inflammation is a major contributor to physiological changes and airway remodeling in asthma. Eosinophils are present and subsequently activated locally within asthmatic airways, and are increased in number when asthma is uncontrolled [77] or severe [78], while being decreased in controlled asthma [79]. Despite numerous murine studies





**Figure 2.** Immune mechanisms of TSLP in asthma relevant to allergic eosinophilic inflammation. TSLP, released in response to allergens, upregulates expression of MHCII and co-stimulatory molecules, facilitating antigen presentation by dendritic cells to CD4<sup>+</sup> naive T cells, and induces upregulation of OX40 L expression on dendritic cells, accelerating differentiation of CD4<sup>+</sup> naive T cells to Th2 cells. It is hypothesized that TSLP can also promote proliferation and differentiation of naive T cells directly. Th2 cells produce IL-4, IL-5 and IL-13, leading to IgE switching in B cells, degranulation of mast cells, airway eosinophilia, mucus hypersecretion from goblet cells, and smooth muscle contraction resulting in airway hyperresponsiveness. TSLP primes recruitment of primitive CD34<sup>+</sup> hemopoietic progenitors from bone marrow to airway tissue and drives local differentiation to mature eosinophils. TSLP can also directly induce mast cells to produce T2 cytokines, and mast cells themselves can produce significant amounts of TSLP following IgE cross-linking. Basophils also release T2 cytokines and histamine in response to TSLP. IgE, immunoglobulin E; IL, interleukin; ILC2, group 2 innate lymphoid cell; MHCII, major histocompatibility complex class II; OX40 L, OX40 ligand; TCR, T cell receptor; Th, T helper; TSLP, thymic stromal lymphopoietin.

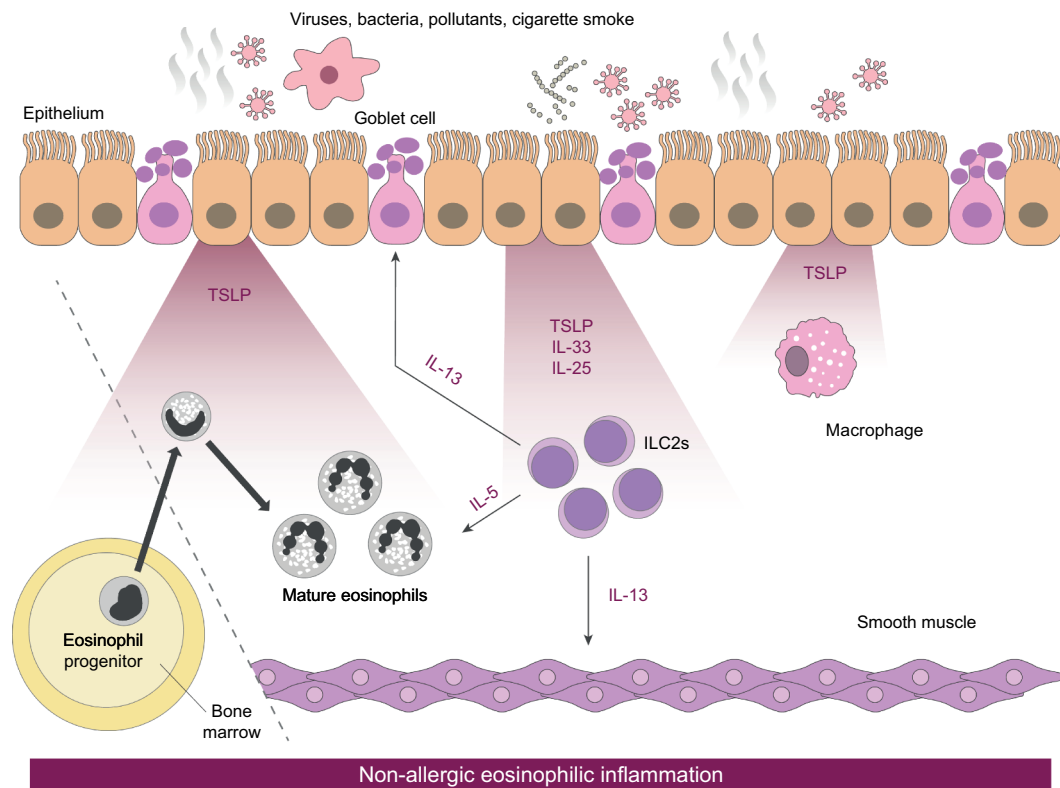
reporting effects of TSLP on eosinophil function, few studies have looked at the direct effect of TSLP on mature human eosinophils, much less a cross-sectional comparison with cells from patients with asthma compared with healthy controls. Human eosinophils express both TSLPR and IL-7Ra subunits, and their expression is enhanced by TNF- $\alpha$  and IL-3 [80,81]. TSLP promotes eosinophil viability by attenuating apoptosis and induces significant production of IL-6, eosinophil-derived neurotoxin and chemokines, including CXCL8, CXCL1 and CCL2 [80,81]. TSLP upregulates ICAM-1 and CD18 but suppresses L-selectin surface expression, indicating that it plays a role in promoting eosinophil transmigration and tissue accumulation [80]. The effects of TSLP on eosinophils are mediated through ERK, p38 MAPK and NF- $\kappa$ B signaling pathways [80,81]. Furthermore, TSLP can induce formation of eosinophilic extracellular traps consisting of mitochondrial DNA in association with eosinophilic cationic protein, which play an important role in innate immune responses to infectious agents leading subsequently to tissue damage in asthmatic airways [82]. These studies indicate a role for TSLP in promoting airway eosinophilia in asthma, and are supported by findings from a clinical trial in patients with mild asthma in which anti-TSLP therapy significantly reduced numbers of blood and sputum eosinophils in conjunction with a reduction in airway bronchoconstriction following allergen challenge [46]. Further

support comes from correlational studies in patients with atopic asthma showing that levels of immunopositive staining for TSLP in bronchial biopsies correlated with airway eosinophilia 24 hours post-allergen challenge [28]. In contrast, levels of TSLP were inversely related to the number of eosinophils in induced sputum from patients with asthma during virus-induced exacerbations, suggesting differing mechanisms of action of TSLP in acute exacerbations versus chronic eosinophilic inflammation [36].

#### 2.4. TSLP and basophils

Basophils play an important role in asthma as a significant source of T2 cytokines, including IL-4, IL-13 and pro-inflammatory mediators such as histamine and leukotrienes. Basophil development, homeostasis and function have been thought to be largely regulated by IL-3; however, accumulating evidence suggests that TSLP also influences basophil differentiation.

Peripheral blood-derived CD34<sup>+</sup> cells pre-incubated with IL-3 and TNF- $\alpha$  have enhanced sensitivity to TSLP-mediated basophil lineage commitment [73]. Additionally, mast cell-activated bone marrow mesenchymal stromal cells produce TSLP, which can enhance differentiation of CD34<sup>+</sup> progenitors into Eo/Bo-CFUs [83]. Mature basophils express TSLPR, which



**Figure 3.** Immune mechanisms of TSLP in asthma relevant to non-allergic eosinophilic inflammation. Exposure to viruses, bacteria, air pollutants, cigarette smoke and other insults induces the release of TSLP and other epithelial cytokines, IL-33 and IL-25, which activate ILC2s. Activated ILC2s produce IL-5 and IL-13, leading to eosinophilia, mucus hypersecretion and airway hyperresponsiveness. TSLP may also have direct effects on eosinophils, promoting eosinophil viability by attenuating apoptosis, as well as priming recruitment of primitive CD34+ hemopoietic progenitors from bone marrow to airway tissue and driving local differentiation to mature eosinophils. Furthermore, TSLP may have effects on macrophages, although these have not yet been fully elucidated. IL, interleukin; ILC2, group 2 innate lymphoid cell; TSLP, thymic stromal lymphopoietin.

can be upregulated in the presence of IL-3 [84]. By comparison, TSLP-stimulated basophils exhibited a greater expression of the IL-33 receptor ST2, suggesting the existence of heterogeneous basophil populations [84]. Allergen stimulation of peripheral blood mononuclear cells in patients with atopic dermatitis resulted in upregulation of TSLPR on basophils and myeloid dendritic cells, which was further increased with IgE-FcεR1 cross-linking [85]. In blood samples from patients with allergic asthma, there was significant upregulation of TSLPR on basophils following direct stimulation with cross-linking anti-IgE antibody, which correlated with serum total IgE [86]. However, another study of patients with asthma reported that anti-IgE stimulation increased IL-25 and IL-33 receptor expression, but not TSLPR [87]. These studies suggest that there may be both IgE-dependent and IgE-independent mechanisms that enhance the responsiveness of basophils to TSLP. Salter *et al.* (2015) expanded on these findings to show that basophil TSLPR expression is increased significantly post-allergen challenge within the airways of individuals with mild asthma [88]. In addition, TSLP stimulation of peripheral basophils increased activation marker expression (CD203 c), T2 cytokine production, histamine release and eotaxin-induced cellular migrational responses [88]. Stimulation of basophils with TSLP also upregulates expression of the IL-25 receptor (IL-17RB) and ST2, suggesting that TSLP can enhance basophil responsiveness to other alarmin cytokines [89]. TSLP is an

important mediator of basophil inflammatory function, and this axis may be a potential target to attenuate airway eosinophilia.

## 2.5. TSLP and mast cells

Mast cells play an important role in initiating eosinophilic and/or allergic asthma through IgE-FcεR1 cross-linking, leading to degranulation of histamine, leukotrienes and cytokines/chemokines. Evidence demonstrates that alarmin cytokines can influence mast cell function. Mast cells express TSLPR and when stimulated with TSLP, alone or in concert with IL-1β and TNF-α, produce T2 cytokines and chemokines CXCL8 and CCL1 with no effect on mast cell proliferation or survival [18,24,90]. Interestingly, mast cells themselves can produce significant amounts of TSLP following IgE cross-linking or priming with IL-4 [91,92], and a crosstalk between ASMCS and mast cells has been reported, as shown by chronically activated mast cells triggering the release of TSLP in a TNF-α-dependent pathway. In turn, ASMC-derived TSLP induced T2 cytokine production by mast cells [93]. Collectively, these studies demonstrate that TSLP can directly interact with mast cells to propagate eosinophilic and allergic inflammation, through the production of T2 cytokines. As mast cells can themselves produce TSLP, there may be an autocrine feedback loop that could be a viable target for asthma management.

## 2.6. TSLP and monocytes/macrophages

Macrophages are an abundant leukocyte found in alveoli, distal airspaces and conducting airways. T2 cytokines can drive differentiation of lung macrophages into alternatively activated macrophages (aAMs). However, there are few supporting studies in humans showing that effects of TSLP directly promote quiescent macrophage differentiation into aAMs. TSLP has been shown to enhance CD80 activation marker expression in blood CD14<sup>+</sup> monocytes/macrophages, indicating a role in promoting differentiation to mature macrophages [94]. In addition, cDNA taken from human monocytes cultured with TSLP and IL-7 showed upregulation of *CCL17*, *CCL18* and *CCL22*, thereby implicating TSLP as a promotor of subsequent migration of effector cells to the airways [95]. Further studies are needed to determine whether TSLP can influence differentiation of macrophages into aAMs in humans. Immunostaining of bronchial biopsy tissue shows that TSLP expression in tissue colocalizes to epithelial CD68<sup>+</sup> macrophages, with greater numbers detected in patients with asthma compared with disease controls or healthy individuals, which supports this postulate [25,29,30].

## 3. Adaptive immune mechanisms of action of TSLP relevant to T2-driven (eosinophilic/allergic) inflammation in asthma

### 3.1. TSLP and dendritic cells

Human myeloid dendritic cells express TSLPR [96], and TSLP stimulation can directly upregulate expression of major histocompatibility complex class II and co-stimulatory molecules CD40, CD86, CD54, CD90, CD83 and CD-LAMP, as well as chemokines CXCL8, CCL24, CCL17, CCL22 and CCL1 [19,97–99]. Interestingly, monocyte-derived dendritic cells can themselves produce TSLP upon stimulation by microbial products, suggesting that TSLP can act in an autocrine manner to further drive T2 inflammation [100,101]. Studies also implicate TSLP as being an important driver of dendritic cell-mediated T cell differentiation [102]. In the absence of IL-12, TSLP can induce expression of OX40 ligand (OX40 L) [98], and OX40 L expressed by TSLP-induced dendritic cells results in differentiation of naive CD4<sup>+</sup> T cells into TNF- $\alpha$ /IL-10<sup>+</sup> T helper (Th) 2 cells [98]. OX40 L expression can convert IL-10-producing regulatory Th1 cells induced by IL-12 into TNF- $\alpha$ -producing Th2 cells, indicating that OX40 L produced by TSLP-stimulated dendritic cells can act as a Th2-polarizing signal [98,103]. Similarly, a combination of TSLP and allergen can stimulate peripheral myeloid dendritic cells from individuals with allergic asthma to induce CD4<sup>+</sup> T-cell differentiation into Th2 cells, whereas TSLP on its own can promote polarization into Th9 cells [104]. OX40 L expression is required for the induction of Th2 but not Th9 polarization, and in contrast Th9 cells require the presence of TGF- $\beta$ 1 [104]. Huang *et al.* (2019) expanded on the above findings to show that exosomes produced by TSLP-activated dendritic cells expressed OX40 L, which had the capacity to promote CD4<sup>+</sup> T-cell proliferation and production of IL-4 [105]. TSLP was shown to have a priming effect on myeloid dendritic cell-mediated expansion and function of

CRTH2<sup>+</sup>CD4<sup>+</sup> Th2 memory cells but directly hindered the development of FOXP3<sup>+</sup>Tregs [106,107]. These studies demonstrate that the interaction between dendritic cells and TSLP is an important triggering event that leads to the promotion of naive T-cell differentiation and polarization, and downstream T2 inflammation, in part mediated by OX40 L.

### 3.2. TSLP and lymphocytes

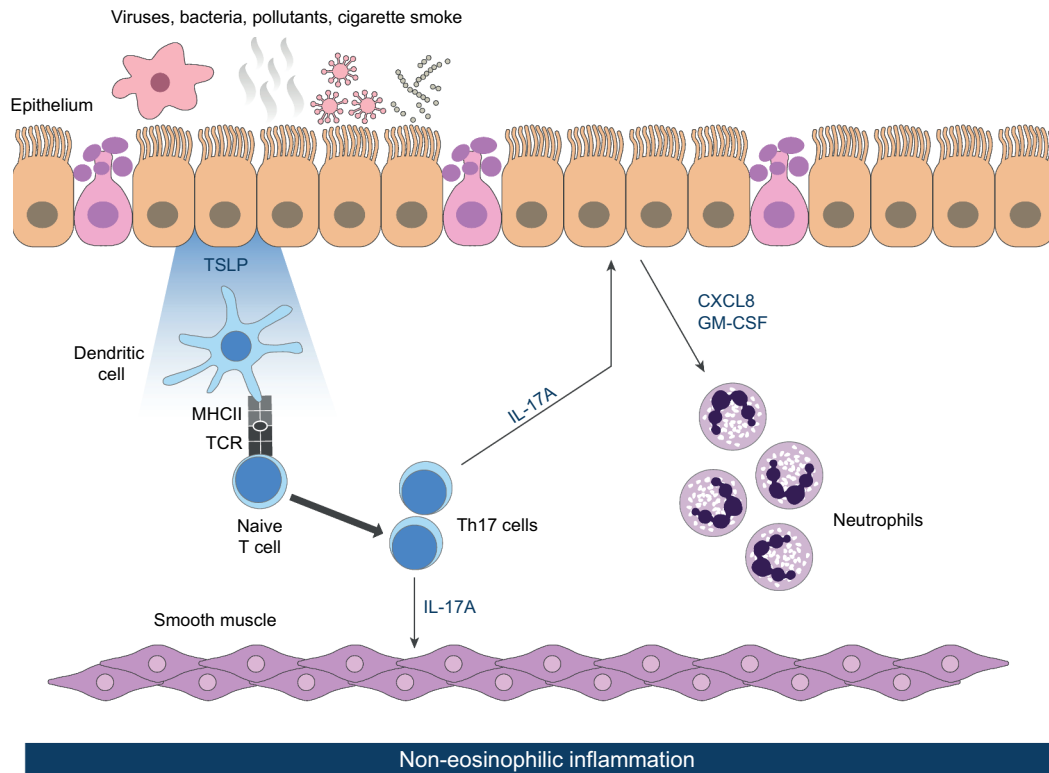
Although the majority of studies investigating TSLP and lymphocytes have focused on the indirect effect of TSLP on T-cell differentiation mediated by dendritic cells [98,102,104], there is evidence to suggest that TSLP can directly modulate human T lymphocytes. In resting CD4<sup>+</sup> T cells, there is minimal expression of TSLPR; however, following their activation, TSLPR expression levels increase significantly [108]. TSLP, in the presence of T cell receptor (TCR) stimulation or IL-4, can promote proliferation and differentiation of naive CD4<sup>+</sup> T cells into Th2 cells or memory T cells [108,109]. Similar effects are seen on CD8<sup>+</sup> T cells: TSLP can directly enhance the expansion of CD8<sup>+</sup> T cells activated with TCR stimulation [110].

The direct effects of TSLP on Tregs have not been well studied. Tregs express TSLPR and stimulation with TSLP impairs IL-10 production [106]. Suppressive Treg activity was found to be decreased in allergic asthmatics compared with non-allergic asthmatics or healthy controls in both adult and pediatric populations [32,106]. These findings suggest that TSLP has the ability to reduce the anti-inflammatory function of Tregs and hence further potentiate T2 inflammation in asthma. Collectively, these data suggest that TSLP can directly modulate T lymphocytes, leading to downstream T2 inflammation and airway eosinophilia.

## 4. Mechanisms of action of TSLP relevant to non-eosinophilic/non-allergic asthma

Asthma is a heterogeneous disease with numerous phenotypes. Moderate and severe asthma phenotypes have been associated with non-eosinophilic inflammation, mediated by Th17 cells and neutrophils (Figures 1 and 4). IL-17A produced by Th17 cells has been shown to have various effects in asthma pathophysiology, including stimulating bronchial epithelial cells to produce neutrophilia-promoting cytokines such as CXCL8 (IL-8) and GM-CSF [111], and promoting airway remodeling by altering the function of ASMCS [112]. However, few studies have investigated the role of TSLP in non-eosinophilic asthma. One study reported that TSLP enhanced Toll-like receptor (TLR) 3 ligand-induced production of IL-23 by dendritic cells, and induced the programming of naive CD4<sup>+</sup> T cells into Th17 cells [113]. Another study reported that TSLP-stimulated dendritic cells pulsed with ovalbumin led to Th2 and Th17 polarization, as evident by an increase in IL-4<sup>+</sup>/IL-17A<sup>+</sup> T cells and upregulation of IL-4/IL-17A protein levels in co-culture supernatant. Furthermore, Th17-related cytokines, like IL-6 and IL-23, were upregulated in co-culture supernatants of TSLP-stimulated dendritic cells pulsed with ovalbumin, compared with those of lipopolysaccharide-stimulated dendritic cells [114]. These findings suggest that TSLP and TLR3 ligands promote Th17-cell differentiation under Th2 polarizing





**Figure 4.** Immune mechanisms of TSLP in asthma relevant to non-eosinophilic inflammation. Exposure to environmental insults leads to airway neutrophilia. The mechanisms by which TSLP acts in this are not yet well understood but may involve Th17 cell differentiation through TSLP-promoted dendritic cell activation and subsequent effects on neutrophils via IL-17A production. IL-17A stimulates bronchial epithelial cells to produce neutrophilia-promoting cytokines such as CXCL8 (IL-8) and GM-CSF, and promotes airway remodeling by altering the function of airway smooth muscle cells. CXCL8, C-X-C motif chemokine ligand 8; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; MHCII, major histocompatibility complex class II; TCR, T cell receptor; Th, T helper; TSLP, thymic stromal lymphopoietin.

conditions through dendritic cell activation. Whether TSLP has a direct effect on Th17 activation and differentiation is unclear. TSLP also has the ability to enhance neutrophil killing of methicillin-resistant *Staphylococcus aureus* (MRSA) during *in vivo* skin infection, directly engaging the complement C5 system to modulate neutrophil reactive oxygen species production [115]. The researchers concluded that TSLP increases MRSA killing in a neutrophil- and complement-dependent manner, suggesting a link between TSLP and an innate immune response [115].

With respect to *in vivo* studies, Li *et al.* (2018) assessed the levels of alarmin cytokines in bronchoalveolar lavage fluid from individuals with asthma at different levels of severity and from healthy control individuals [35]. The concentrations of IL-33 and TSLP, but not IL-25, were significantly greater in those with asthma compared with controls, and these cytokine levels correlated inversely with lung function. Further, the concentration of TSLP alone correlated positively with neutrophil counts. Previous studies have shown that neutrophils are a source of TSLP in bronchial biopsy tissue [29,30,116], and this may explain the findings of Corren *et al.* (2017), who reported that anti-TSLP monoclonal antibody therapy reduced exacerbations in patients with severe asthma without blood eosinophilia. The authors proposed that TSLP may play a role in patients with low or absent T2 inflammation [47]. The prevalence of TSLP in other airway diseases, such as chronic obstructive pulmonary disease

[30], further suggests that TSLP may be involved in other T2-independent inflammatory pathways.

## 5. Structural mechanisms of action of TSLP relevant to asthma

Further to its actions on specific immune cells, there is now substantial evidence that TSLP serves as a key mediator between immune cells and structural cells in the airway (Figures 1 and 5). Dysregulation of structural cells in asthma can result in characteristic alterations to the airway, collectively known as airway remodeling, which include thickening of the reticular basement membrane, goblet cell hyperplasia, subepithelial fibrosis and ASMC hyperplasia and/or hypertrophy [117].

### 5.1. TSLP and ASMCs

Numerous reports indicate that TSLP is an important modulator of ASMC activity. Human ASMCs express TSLPR [24], and stimulation with TSLP results in expression of IL-6, CCL11 and CXCL8, as well as migration through STAT3 signaling [118–122]. ASMCs are a significant source of TSLP [24,119,123], augmented in the presence of TNF- $\alpha$  and IL-1 $\beta$  via the p38 and MAPK signaling pathways [118,124]. Allakhverdi *et al.* (2009) showed that TNF- $\alpha$  and IL-1 $\beta$  can promote TSLP expression in ASMCs from healthy individuals,

and that supernatants of IgE/anti-IgE-activated mast cells induced TSLP release in ASMCs [93]. Cultures of supernatants of IL-1- and TNF- $\alpha$ -stimulated ASMCs triggered release of IL-5 and IL-13 by mast cells, which was attenuated by TSLP blockade [93]. Collectively, these findings indicate that TSLP can promote airway inflammation through a crosstalk between mast cells and airway structural cells, as well as communication between the airway epithelium and mast cells [18,24,30,93].

## 5.2. TSLP and fibroblasts

Studies using a human lung fibroblast cell line co-cultured with epithelial cells transfected with TSLP demonstrated significant production of collagen and alpha smooth muscle actin via a p38-MAPK- and STAT3-dependent pathway [125,126]. This indicates that TSLP released by lung epithelial cells in airway diseases such as asthma may promote airway remodeling through activation of fibroblasts. Furthermore, expression of TSLP in bronchial biopsy tissue has been shown to be localized to fibroblasts [116,123,127]. Specifically, TSLP has been shown to increase TGF- $\beta$ 1 and arginase 1 production by fibroblasts at the mRNA and protein levels [128]. It has been proposed that TSLP stimulation can induce fibroblast cellular senescence during airway remodeling in asthma and that inhibiting the signaling pathways of senescence overcomes TSLP-induced airway remodeling [129]. Mechanistic

data from clinical studies with biologics targeting TSLP function will be required to corroborate these *in vitro* findings.

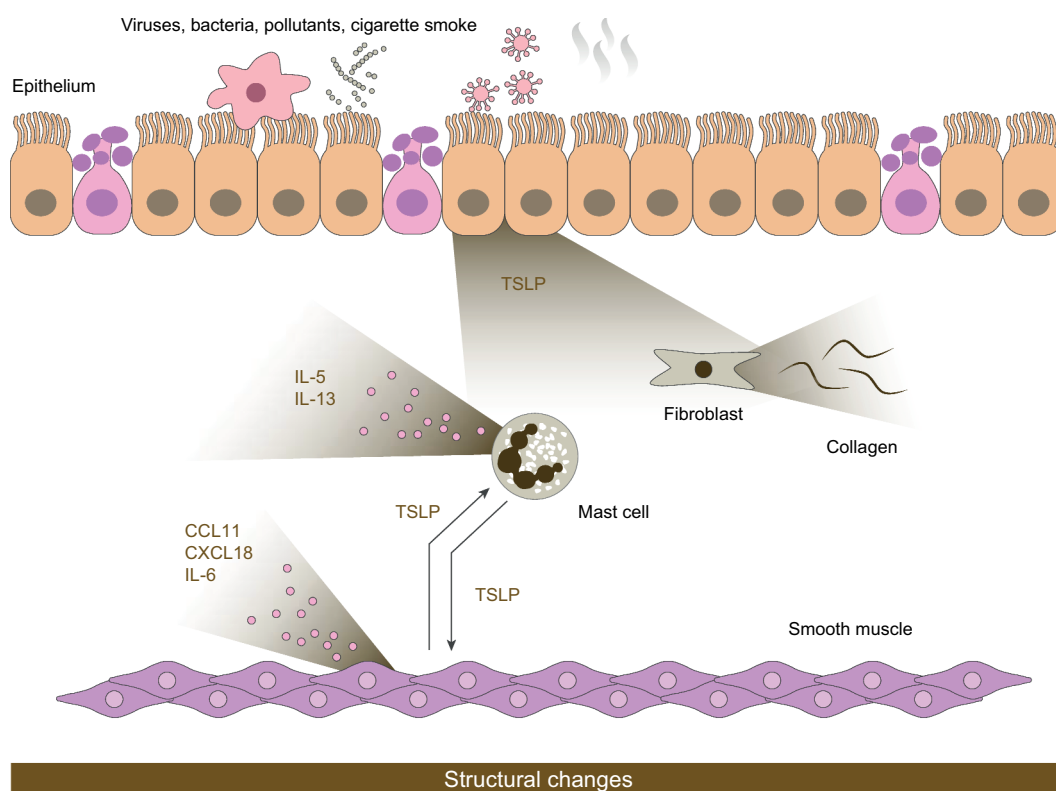
## 6. Clinical data with TSLP blockade

As described above, there is extensive evidence that TSLP plays a major role in the pathophysiology of asthma, based on its position at the top of the inflammatory cascade for both T2 and non-T2 inflammatory processes. Blockade of TSLP may therefore be effective for a broad population of patients with severe, uncontrolled asthma and thus has been actively pursued as a therapeutic treatment.

### 6.1. Drugs in development

There are two investigational medications in clinical development for the treatment of asthma that directly bind to TSLP. The first of the investigational medications to be tested in asthma is tezepelumab, a human monoclonal antibody that binds to TSLP and thus prevents binding and signaling through the TSLPR. Tezepelumab was initially tested as an intravenous formulation and is more recently being tested with subcutaneous delivery.

A second investigational anti-TSLP medication to be tested in asthma is CSJ117, a fully human neutralizing antibody antigen-binding fragment (Fab) that belongs to the IgG1/ $\lambda$  isotype subclass. CSJ117 has been developed as an inhaled formulation for targeted delivery to the lungs to bind to the TSLP



**Figure 5.** Structural mechanisms of TSLP relevant to multiple asthma inflammatory endotypes. These mechanisms include stimulating airway smooth muscle cell migration and mediating crosstalk between airway smooth muscle cells and mast cells, inducing both cell types to produce TSLP and inflammatory cytokines. TSLP also stimulates human lung fibroblast cells to produce collagen, promoting airway remodeling. CCL11, C-C motif chemokine ligand 11; CXCL8, C-X-C motif chemokine ligand 8; IL, interleukin; TSLP, thymic stromal lymphopoietin.

released by airway epithelial cells in response to common inhaled triggers [16,17]. This formulation has potentially greater convenience than the systemic delivery of tezepelumab, while directly targeting the lung epithelium via local distribution of drug may potentially reduce the chance of effects caused by impacting TSLP signaling outside the lung.

The TSLPR complex has been associated with a number of allergic inflammatory diseases in addition to asthma [130]. GSK2618960 is a humanized Fc-disabled IgG1 monoclonal antibody directed against the alpha component (IL-7R $\alpha$ ; CD127) of the TSLPR [131] and is currently in development for the treatment of autoimmune indications including multiple sclerosis [132]. Together with evidence of autoantibodies in airways of patients with severe eosinophilic asthma [133], blocking IL-7R $\alpha$  may have utility for the treatment of asthma. Administered intravenously, this investigational medication has been well tolerated. By flow cytometry, GSK2618960 demonstrated greater than 95% receptor occupancy on CD3<sup>+</sup> T cells and effectively blocked IL-7 receptor signaling as measured by STAT5 phosphorylation following *ex vivo* exposure of whole blood to IL-7 stimulant [132].

## 6.2. Clinical studies of TSLP blockade

Two clinical trials of TSLP blockade in patients with asthma have been published, reporting favorable results of tezepelumab treatment. Results from a trial of CSJ117 are pending. Completed and ongoing clinical studies of TSLP blockade are summarized in Table 2.

The first trial completed in patients with asthma was a phase 1b, proof-of-concept study to evaluate the efficacy of tezepelumab in an allergen challenge model of allergic asthma. This randomized, parallel-group, double-blind, placebo-controlled study was conducted by the Clinical Investigators Collaboration in a Canadian population of adults with mild, allergic asthma [46]. Tezepelumab was administered intravenously at a dose of 700 mg and participants were dosed every 4 weeks for 3 months. In the tezepelumab group, blood eosinophil counts began to decline at 2 weeks post-dosing (the first time point measured) and reached normal levels by 4 weeks. Sputum eosinophils showed a significant improvement into the normal range (of < 2%) by the first time point measured, 6 weeks after the first dose. Remarkably, the level of fractional exhaled nitric oxide (FeNO) improved significantly by 1 week after the first dose. Inhaled allergen challenges were conducted on days 42 and 84 to induce eosinophilic inflammation in the airways; tezepelumab significantly inhibited the allergen-induced early and late asthmatic responses, as well as post-challenge measures of inflammation, including FeNO, and eosinophils in blood and sputum. It was noted that the systemic treatment was effective in regulating both circulating and local measures of inflammation.

The second completed trial in asthma (PATHWAY) was a large, phase 2, multicenter, randomized, parallel-group, double-blind, placebo-controlled study [47]. The trial evaluated the efficacy and safety of tezepelumab as an add-on therapy for patients with moderate-to-severe asthma and a history of exacerbations and uncontrolled disease, who

were receiving inhaled corticosteroids and long-acting  $\beta_2$ -agonists with or without oral corticosteroids and additional asthma controllers. Three tezepelumab dose regimens were evaluated, low (70 mg every 4 weeks), medium (210 mg every 4 weeks) and high (280 mg every 2 weeks), administered subcutaneously for 1 year. The study reported significant reductions versus placebo in annualized exacerbation rates of 62%, 71% and 66% in the low-, medium- and high-dose tezepelumab groups, respectively, along with significant improvements in lung function and markers of inflammation (FeNO and blood eosinophils) in all active treatment groups. Of interest, these improvements were observed irrespective of patient phenotype and independent of baseline peripheral blood eosinophil counts, IgE levels and FeNO levels, indicating that tezepelumab provided similar efficacy in patients with T2-driven or non-T2-driven disease. Assessments of pro-inflammatory biomarkers and proteomics were also conducted. In the cohort receiving tezepelumab 210 mg every 4 weeks (the dose selected for phase 3 studies), serum IL-5 and IL-13 levels and numbers of blood eosinophils at 1 year decreased by at least 50% from baseline, along with 25% and 20% reductions in FeNO and total IgE, respectively [134]. Proteomics analyses revealed reductions in proteins associated with matrix remodeling (MMP-10 and periostin) demonstrating broad biological effects of TSLP blockade [135].

In addition to the studies of tezepelumab, a multinational proof-of-concept study of CSJ117 in the allergen challenge model with patients with mild, allergic asthma has been performed to evaluate safety, tolerability, pharmacokinetics and pharmacodynamics. Twenty-eight participants completed the study, which comprised daily inhalation of CSJ117 and allergen challenges conducted at 6 and 12 weeks. The study was completed in 2019 with results pending [136].

Further clinical trials to assess the efficacy, mechanisms and long-term safety of tezepelumab are underway. Two pivotal phase 3 studies (NAVIGATOR and SOURCE) are being conducted in patients with severe asthma who are receiving inhaled corticosteroids/long-acting  $\beta_2$ -agonists with or without maintenance oral corticosteroids and additional asthma controllers [137,138]. The primary outcomes are reductions in asthma exacerbation rate and daily oral corticosteroids, respectively. An additional bronchoscopy study (CASCADE) aims to improve understanding of the mechanisms of TSLP blockade by assessing the effects of tezepelumab on the number of inflammatory cells in endobronchial biopsies collected from adults with inadequately controlled, moderate-to-severe asthma [139]. Data regarding long-term safety and tolerability will be important and are currently being addressed in a tezepelumab extension trial (DESTINATION) [140]. Together, these studies will provide much-needed information regarding the benefits of blocking TSLP in asthma.

## 7. Conclusion

There is substantial evidence that TSLP plays a central role in epithelial-driven inflammation, beginning with the response to environmental insults and leading to multiple innate and

**Table 2.** Clinical studies of TSLP blockade: completed and ongoing studies in patients with asthma.

Study number	Drug	Estimated start and completion dates	Patient population	Phase	Primary outcome
NCT00972179	Tezepelumab (TSLP mAb)	Completed 2010	49 healthy volunteers	1	Safety of SC and IV doses
NCT00757042	Tezepelumab (TSLP mAb)	Completed 2011	78 healthy volunteers and individuals with moderate-to-severe atopic dermatitis	1	Safety of SC and IV doses
NCT01405963	Tezepelumab (TSLP mAb)	Completed 2013	31 adults with mild allergic asthma	1	Allergen-induced late asthmatic response
NCT01913028	Tezepelumab (TSLP mAb)	Completed 2014	24 adult healthy Japanese men	1	safety
Safety and tolerability of SC doses					
NCT02512900	Tezepelumab (TSLP mAb)	Completed 2016	21 adolescents with mild-to-moderate asthma	1	safety
Pharmacokinetic profile of SC doses					
NCT02054130 (PATHWAY)	Tezepelumab (TSLP mAb)	Completed 2017	584 adults with uncontrolled, moderate-to-severe asthma	2	Annualized asthma exacerbation rate
NCT02237196 (CATNIP)	Tezepelumab (TSLP mAb)	2015–2019	121 adults with moderate-to-severe allergic rhinitis	1/2	Allergen-induced total nasal symptom score
NCT02698501 (UPSTREAM)	Tezepelumab (TSLP mAb)	2016–2019	40 adults with asthma requiring ICS ( $\pm$ LABA)	2	Mannitol PD15
NCT03989544 (PATH-BRIDGE)	Tezepelumab (TSLP mAb)	2019–2019	315 healthy adults	1	Pharmacokinetics of SC administration via accessorized pre-filled syringe or autoinjector compared with vial and syringe
NCT03968978 (PATH-HOME)	Tezepelumab (TSLP mAb)	2019–2020	216 adults and adolescents with severe asthma	3	Successful SC administration via accessorized pre-filled syringe or autoinjector at home versus in the clinic
NCT03347279 (NAVIGATOR)	Tezepelumab (TSLP mAb)	2019–2020	1038 adults and adolescents with severe, uncontrolled asthma taking medium-to-high-dose ICS and at least one additional asthma controller with or without OCS	3	Annualized asthma exacerbation rate
NCT03406078 (SOURCE)	Tezepelumab (TSLP mAb)	2018–2020	150 adults with oral corticosteroid-dependent asthma (Americas, Europe)	3	Reduction in daily OCS dose
NCT03688074 (CASCADE)	Tezepelumab (TSLP mAb)	2018–2020	116 adults with inadequately controlled moderate-to-severe asthma, taking ICS and at least one additional asthma controller	2	Number of airway submucosal inflammatory cells/mm <sup>2</sup> of bronchoscopic biopsies
NCT03706079 (DESTINATION)	Tezepelumab (TSLP mAb)	2019–2022	966 adults and adolescents with severe, uncontrolled asthma	3	Exposure-adjusted incidences of adverse events and serious adverse events
NCT04048343 (NOZOMI)	Tezepelumab (TSLP mAb)	2019–2021	66 Japanese adults and adolescents with inadequately controlled severe asthma	3	Rate of adverse events
NCT03927157 (DIRECTION)	Tezepelumab (TSLP mAb)	2019–2023	396 Chinese adults with severe, uncontrolled asthma taking medium-to-high-dose ICS and at least one additional asthma controller with or without OCS	3	Annualized asthma exacerbation rate
NCT03138811	CSJ117 (TSLP mAb fragment)	2017–2019	28 adults with mild, stable, atopic asthma	1	Allergen-induced late asthmatic response

ICS, inhaled corticosteroids; IV, intravenous; LABA, long-acting  $\beta_2$ -agonist; mAb, monoclonal antibody; OCS, oral corticosteroids; SC, subcutaneous; TSLP, thymic stromal lymphopoietin.

adaptive inflammatory pathways. While it is well established that TSLP drives T2 inflammation following its release from the epithelium, there is growing evidence that TSLP also plays a role in non-T2 processes involving both immune and structural cells. Myriad effects of TSLP have been identified on a variety of cell types including ILC2s, hematopoietic progenitor cells, eosinophils, basophils, mast cells, monocytes/macrophages, dendritic cells, lymphocytes, neutrophils, smooth muscle cells and fibroblasts.

In patients with asthma, TSLP production appears to be dysregulated, being overexpressed compared with healthy individuals and correlating with asthma severity and airway obstruction. Elements of asthma pathophysiology, including airway hyperresponsiveness, mucus overproduction and airway remodeling, are

at least partly driven by TSLP via its downstream, pro-inflammatory effects. The position of TSLP at the top of the inflammatory cascade makes it an attractive therapeutic target. Clinical trials of systemic TSLP blockade with tezepelumab in patients with asthma have yielded promising results, including significant reductions in exacerbation rates, improvements in lung function and reductions in multiple biomarkers of inflammation.

## 8. Expert opinion

Treatment options for asthma have rapidly expanded in recent years with the introduction of biologics developed for specific patient endotypes. The aim of these new medications is to control a specific inflammatory pathway that is dysregulated (i.e. IgE,



IL-5, IL-4/IL-13), and subsequently improve asthma control and prevent exacerbations. By activating a range of downstream inflammatory pathways, TSLP affects disease activity more broadly than a single downstream pathway. As TSLP expression in the airway is abnormally elevated in patients with asthma, TSLP blockade may be considered to have an immunomodulatory function, restoring homeostatic balance to the airways.

The results of clinical trials provide the strongest evidence of the importance of TSLP in driving asthma. Blocking TSLP is the first biological approach that has been shown to produce clinically meaningful reductions in blood eosinophils, circulating IgE, and FeNO. The airway epithelium is the site of a variety of asthma triggers, including viruses, allergens, bacteria and fungi, so targeting this compartment may be effective in treating asthma caused by multiple triggers. TSLP blockade has been shown to be a promising approach for treating both T2-driven and non-T2-driven (i.e. non-allergic, non-eosinophilic) inflammation in asthma when dosed for periods of up to 1 year. There are limited therapeutic options for patients with non-T2-driven inflammation, which is characterized by either neutrophilic or paucigranulocytic airway inflammation. Although the disease mechanisms of these asthma endotypes are not well understood, it is noteworthy that TSLP blockade has been shown to be effective in this population of patients. The potential glucocorticoid-sparing effects of TSLP blockade represents another important area of study.

As with other biologics, it will be important to investigate biomarkers to identify patients who best respond to anti-TSLP therapy. Blood eosinophils, serum IgE and FeNO have been used as biomarkers to guide treatment with anti-IL-5/IL-5R $\alpha$ , anti-IgE and anti-IL-4/IL-13 monoclonal antibodies in severe asthma. While TSLP itself could hypothetically be used as a biomarker to identify patients with elevated levels, this has not been established largely owing to difficulties in accurately identifying and measuring low concentrations of this cytokine. Furthermore, in nasal polyp tissues, it has been shown that TSLP can be cleaved by endogenous proteases to generate bioactive peptides [141,142], which may contribute to lack of detection by anti-TSLP antibodies in *ex vivo* work and may also result in underestimation of actual TSLP production. The clinical relevance of systemic detection of TSLP is also unclear, as studies examining associations between TSLP expression and disease manifestations have examined airway levels of TSLP. Additionally, the episodic expression of TSLP protein in response to various triggers means that TSLP levels in circulation may not fully reflect the local tissue environment during an asthma exacerbation.

Attempts to quantify TSLP in patient samples are also complicated by the existence of two isoforms of the protein: the full-length protein, often called long-form TSLP (lTSLP), and a form comprising about half the amino acid length (63-amino-acid acids), often called short-form TSLP (sTSLP) [143–146]. It is not currently known if the anti-TSLP therapies in clinical development bind to the lTSLP, sTSLP, or both. While the role of lTSLP has been well characterized, the function of sTSLP remains uncertain. It is believed to be constitutively expressed in human tissues but not in rodents [147,148]. Furthermore, it does not bind to the TSLPR

complex [147,149], suggesting a distinct biological function from lTSLP. The relative ratio of lTSLP to sTSLP has not been established in patients with asthma, owing in part to a lack of available research reagents able to discern between the two forms of TSLP. At present, lTSLP versus sTSLP can only be distinguished at the mRNA level, by using specific primers. Such studies examining the two isoforms in human tissue have revealed that the long isoform of TSLP is pro-inflammatory and is expressed during inflammation [150], and that the TSLP isoform ratio may be altered during several inflammatory disorders [149]. Further research is needed to improve our understanding of the role of the two isoforms of TSLP, their regulation by SNPs and their expression under different pathological conditions.

Although TSLP is primarily expressed by epithelial cells at barrier surfaces (lung, gut, skin), TSLP can be also be produced by a range of immune cells and can thereby contribute to the pathology of asthma at sites distal to the airways, such as the bone marrow for hematopoiesis. As such, systemic dosing of anti-TSLP, blocking TSLP signaling throughout the body, may be an effective approach; however, it also has the potential to disrupt other homeostatic roles of TSLP [149,151]. The long-term safety and efficacy of anti-TSLP treatment therefore needs to be evaluated, ideally considering not only T2-driven and non-T2-driven inflammation, but also TSLP variants, gene polymorphisms and ethnically diverse populations. Furthermore, inhaled TSLP blockade, directly targeting TSLP produced in the airways, is an interesting alternative route being assessed. Future studies evaluating the effects of inhaled TSLP blockade may help us to understand the relative contribution of airway epithelial cell TSLP production to the pathology of asthma, and whether local delivery to the airways is effective and has relevance for the safety and tolerability of anti-TSLP therapy.

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## Declaration of interest

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