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Invited Review Article

Anti-TSLP antibodies: Targeting a master regulator of type 2 immune responses

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ABSTRACT

TSLP is an epithelial cell-derived cytokine synthesized in response to various stimuli, including protease allergens and microorganisms like viruses and bacteria. Biological functions of TSLP require heterodimer formation between the TSLP receptor (TSLPR) and IL-7 receptor- α , which polarize dendritic cells to induce type 2 inflammation and directly expand and/or activate Th2 cells, group 2 innate lymphoid cells, basophils, and other immune cells. TSLP is thus considered a master regulator of type 2 immune responses at the barrier surfaces of skin and the respiratory/gastrointestinal tract. Indeed, genetic, experimental, and clinical evidence suggests that the TSLP-TSLPR pathway is associated with the pathogenesis of allergic diseases such as atopic dermatitis (AD) and asthma. Tezepelumab (AMG-157/MEDI9929) is a human anti-TSLP antibody that prevents TSLP-TSLPR interactions. A phase 2 trial for moderate to severe AD showed that a greater but not statistically significant percentage of tezepelumab-treated patients showed clinical improvements compared to the placebo group. A phase 2 trial for uncontrolled, severe asthma showed significant decreases in asthma exacerbation rate and improved pulmonary function and asthma control for tezepelumab-treated patients. Levels of biomarkers of type 2 inflammation, such as blood/sputum eosinophil counts and fraction of exhaled nitric oxide decreased, however, clinical efficacy was observed irrespective of the baseline levels of these biomarkers. A blockade of the TSLP-TSLPR pathway likely will exert significant clinical effects on AD, asthma, and other allergic diseases. The efficacy of anti-TSLP antibodies compared to other biologics needs to be further examined.

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Introduction

TSLP is a secreted molecule of a murine thymic stromal cell line that was identified more than two decades ago. Since then, a human orthologue has also been identified.^{1,2} TSLP has been widely studied as a master regulator of Th2-type (type 2) immune responses occurring at barrier surfaces such as the skin, lungs, and gut. TSLP produced by epithelial cells at barrier surfaces activates

TSLP receptor (TSLPR)-expressing DCs to induce functional Th2 cells.³ In addition, TSLP is known to be associated with other diseases, including autoimmune disorders and cancer.⁴ Such a broad pathophysiological profile has motivated the therapeutic targeting of TSLP- and TSLPR-mediated signaling in type 2 cytokine-mediated allergic diseases. In this review, we summarize the known molecular mechanism of TSLP-TSLPR-mediated signaling and its pathological roles in atopic dermatitis (AD) and asthma to convey the potential usefulness of the TSLP-TSLPR signaling pathway as a therapeutic target for these diseases.

Biological properties of TSLP and TSLPR

TSLP is a member of the IL-2 cytokine family and was originally classified as a molecule that can stimulate murine thymocytes and promote B cell proliferation and development.² A human homolog was subsequently identified, and further characterization of this

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cytokine revealed a four-helix bundle structure containing six conserved cysteine residues and multiple potential sites for N-linked carbohydrate additions. In spite of only 43% amino acid identity, human and murine TSLP share a significant degree of functional homology.¹

Epithelial cells in the lungs, skin, and gastrointestinal tract are reported to be the primary source of TSLP during homeostatic and inflammatory conditions, although other immune cells such as DCs, basophils, and mast cells can also produce TSLP.^{5–7} In addition, TSLP expression in target cells can be induced by several cytokines, such as TNF- α and IL-1 β ,⁸ respiratory viruses,⁹ bacterial and fungal products,^{10,11} mechanical injury,¹² allergens,¹³ cigarette smoke extracts,¹⁴ and proteases such as tryptase and papain.¹⁵

TSLP binds to a heterodimeric receptor consisting of the IL-7 receptor α -chain (IL-7R α) and the TSLPR chain, which is closely related to the common receptor γ chain (γ C), in order to exert its biological activity on a broad range of cell types. TSLPR alone has a low affinity for TSLP but binding of TSLPR to IL-7R α creates a high affinity binding site for TSLP and triggers signaling.¹⁶ In mice, the TSLP-signal transducer and activator of transcription (STAT) 5 axis in DCs is a critical pathway for promoting type 2 immune responses at barrier surfaces.^{17,18}

TSLP is a cytokine involved in both physiological and pathological immunity-related activities. TSLP has homeostatic activities due to its expression by Hassall corpuscles in the thymus and regulates the capacity of DCs and plasmacytoid DCs to drive development of Tregs.^{19,20} TSLP also promotes homeostatic polyclonal expansion of T cells in the absence of foreign antigens.²¹ In the gut, TSLP constitutively expressed by intestinal epithelial cells can act on non-inflammatory DCs that have reduced abilities to produce IL-12p70²² and drive the differentiation of inducible Tregs.²³ In contrast, in inflammatory conditions, TSLP stimulates DCs to upregulate co-stimulatory molecules like OX40L, CD80, and CD86.⁷ TSLP-treated DCs can drive activity of IL-4, IL-5, and IL-13-producing CD4⁺ T cells *in vitro*. In addition to the effects of TSLP on Th2 cell polarization through antigen-presenting cells,^{24,25} TSLP can directly act on T cells, including CD4⁺ T cells, CD8⁺ T cells, and Tregs. TSLP also plays an essential role in Treg-cell-dependent immune homeostasis in the skin through TSLPR signaling in Treg cells. Loss of TSLPR signaling in skin-associated Tregs inhibits their activation and permits rapid progression from skin pro-inflammatory responses to lethal systemic inflammation.²⁶ TSLP can also promote Th2 cytokine responses through its actions on mast cells, ILCs, epithelial cells, macrophages, and basophils.^{27–31} In addition, TSLP can directly act on a subset of sensory neurons to induce itch in AD.¹⁵ In murine models of allergic disease, TSLP can induce differentiation of Th2 cells and Th9 cells through its effects on DCs and T cells.^{32,33} TSLP signaling in basophils is important in murine models of experimental eosinophilic esophagitis and food allergies.^{34,35}

Two isoforms of TSLP, short and long isoforms, have been described in mice, but the functional consequences of this variation are unclear. In humans, the main isoform expressed during steady state conditions is the short form of TSLP, whereas the long form of TSLP is upregulated in inflammatory conditions.^{36,37} In addition, there is evidence that in pathological conditions, TSLP can be cleaved by several endogenous proteases.^{38,39} For example, in celiac disease patients, the protease furin, which is upregulated in patient biopsies, can cleave the long isoform of TSLP, producing 10 kDa and 4 kDa fragments with different activities on human peripheral blood mononuclear cells compared to mature TSLP.⁴⁰ Cleavage of TSLP by serine proteases may also regulate TSLP protein levels or function in the skin. However, since the expression patterns and biological properties of these two different isoforms of TSLP seem to be distinct, these two TSLP isoforms should be analyzed separately in future studies.

TSLP in AD

AD is a common chronic skin inflammatory disorder characterized by recurrent eczema accompanied by an intractable itch leading to an impaired quality of life. The complex interplay among skin barrier dysfunction, immunological derangement, and pruritus contributes to the development, progression, and chronicity of AD.^{41–43} AD also has a complex etiology, including genetic and environmental factors, that leads to abnormalities in epidermal and immune system function.^{44,45}

TSLP polymorphisms in AD patients

Polymorphisms in the TSLP gene are associated with an increased risk of development and progression of AD. AD is significantly associated with three TSLP-single nucleotide polymorphisms (SNPs) (rs1898671, rs11466749, and rs10043985), four IL7R-SNPs (rs12516866, rs102113865, rs1389832, and rs10058453), and three TSLPR-SNPs (rs36139698, rs36177645, and rs36133495) in European American populations.⁴⁶

The role of TSLP in AD pathogenesis

Blood serum levels of TSLP in children and adults with AD are significantly increased compared to healthy subjects.^{47,48} In addition, TSLP is expressed in keratinocytes of epidermal apical layers in skin lesions of acute and chronic AD patients, but not in non-lesional skin and not in lesions of patients with nickel-induced allergy contact dermatitis or cutaneous lupus erythematosus.⁷

The role of TSLP in acquired immune cells in AD

Induced expression of TSLP in mouse epidermal keratinocytes upon topical application of MC903, a low calcemic analogue of vitamin D3, triggers AD and aggravates experimental allergic asthma, indicating an important role of keratinocyte-produced TSLP in the link between AD and asthma.^{49,50} The significance of TSLP in the pathogenesis of AD has been confirmed using transgenic mice in which selective overexpression of TSLP in the skin caused spontaneous AD-like dermatitis.⁵¹ TSLP-TSLPR in Langerhans cells as antigen-presenting cells in the epidermal signaling pathway is critical for induction of Th2 type immune responses in an ovalbumin application-induced murine model of AD⁵² (Fig. 1). In addition to antigen presenting cells, circulating CD4⁺ T cells in AD patients show high levels of TSLPR expression compared to healthy subjects and the level of circulating TSLPR⁺ CD4⁺ T cells correlates with serum CCL17/TARC and IgE levels⁵³ and eosinophil counts, suggesting that TSLPR is a potential therapeutic target for AD.

TSLP and innate and other cell populations in AD

In addition to acquired immune cell populations, basophils and ILCs are crucial innate cell populations downstream of TSLP.⁵⁴ TSLP promotes peripheral basophilia and TSLPR-expressing basophils can restore Th2-dependent immunity in mice.²⁸ Among ILC subpopulations, group 2 ILCs (ILC2s) are present in healthy human skin and are enriched in lesional skin of AD patients. ILC2s are also found in healthy murine skin and are critical for the development of inflammation in a murine model of AD-like dermatitis induced by MC903 application²⁷ (Fig. 1).

TSLPR and IL-7R α transcripts are expressed in mouse and human dorsal root ganglion cells and TSLPR expression is localized to a subset of primary afferent nerve terminals in mouse skin. Injection of TSLP into mouse cheek skin induced scratching behavior in an IL-7R α and primary afferent neuron-dependent manner. TSLPR activation of primary afferent sensory neurons requires the ion channel transient receptor potential (TRP)A1 but not TRPV1¹⁵

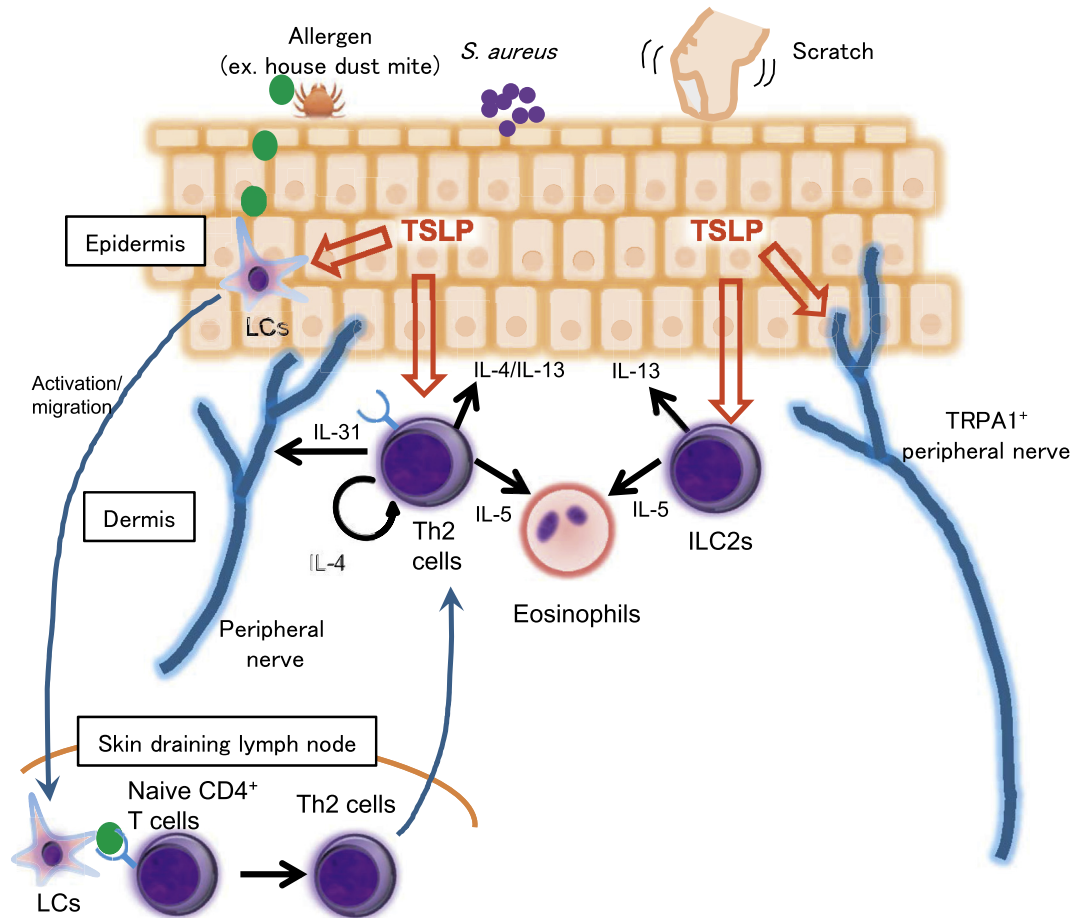


Fig. 1. The role of TSLP in AD pathogenesis. Keratinocytes produce TSLP in response to exposure to allergens such as house dust mite, *S. aureus* proteases, and mechanistic injury caused by scratching. TSLP acts on TSLPR in epidermal LCs to induce Th2 immune responses in the skin, draining the lymph nodes. TSLP also directly acts on Th2 cells to produce Th2 cytokines, such as IL-4, IL-5, IL-13 and IL-31. TSLP acts on ILC2s in the skin to produce Th2 cytokines such as IL-5 and IL-13. Th2 cytokines released in lesional skin provoke pruritus, which induces scratching and the regional production of TSLP. In addition, TSLP directly stimulates TRPA1⁺ sensory nerves to induce itch in AD skin.

(Fig. 1). These data identify TSLP as a novel endogenous pruritogen and suggest that keratinocyte-derived TSLP can be a therapeutic target against pruritus in AD.

Effects of the skin microbiome on TSLP production in AD

Staphylococcus aureus (*S. aureus*) is detected in 90% of lesional skin of patients with AD and is rarely detected in healthy skin⁵⁵. Similarly, analysis of the skin microbiome using next-generation sequencing detected *S. aureus* in patients with AD, and its relative abundance is greatly increased during disease flares compared to baseline conditions,⁵⁶ suggesting that skin inflammation may accelerate the colonization of *S. aureus* or vice versa.⁵⁷

In the context of inflammation promotion during AD, *S. aureus* produces a protease enabling it to penetrate into the dermis of AD patients or mice with filaggrin loss-of-function mutations.^{58,59} *S. aureus* penetration results in increased production of type 2 cytokines like TSLP, IL-4, and IL-13⁵⁸. *S. aureus* cell wall components also signal through toll-like-receptor 2/6 and induce TSLP production in keratinocytes¹⁰ (Fig. 1).

Anti-TSLP antibody therapy for AD

As above described, TSLP plays a critical role in the pathogenesis of AD and atopic march. TSLP is released by epidermal keratinocytes upon various kind of stimulation. In turn, TSLP favors Th2 responses and pruritus pathway activity by affecting a subset of

sensory neurons. TSLP is thus a promising therapeutic target for AD pathogenesis. Tezepelumab (AMG-157/MEDI9929) is a human monoclonal antibody targeting circulating TSLP. Tezepelumab has completed phase 1 (NCT00757042) and phase 2a (NCT02525094) randomized, double-blinded, placebo-controlled studies evaluate its efficacy and safety in subjects with moderate to severe AD. In the phase 2a study (NCT02525094), 113 patients were randomized 1:1 and provided either 280 mg of subcutaneous tezepelumab or a placebo every 2 weeks along with class 3 topical corticosteroids (TCS). The primary endpoint was the week 12 response rate, with a goal of a 50% reduction in the Eczema Area and Severity Index (EASI50). Greater but not statistically significant percentages of tezepelumab plus TCS-treated patients achieved EASI50 (64.7%) versus placebo plus TCS patients (48.2%; $P = 0.091$) (Table 1).⁶⁰ Another study with the anti-TSLP drug MK-8226 was performed in a phase 1b trial (NCT01732510) but was stopped in the middle of the trial and data not reported.

TSLP in asthma

Asthma is a chronic lung disease characterized by chronic airway inflammation, airway hyperresponsiveness, and variable obstruction. The most common molecular mechanism underlying asthma is Th2 (type 2) inflammation mediated by type 2 cytokines, such as IL-4, IL-5, and IL-13, which are mainly derived from Th2 cells and ILC2s. However, some patients with asthma have no

Table 1
Current status of clinical trials for anti-TSLP antibodies.

Compound	Target disease	Indication	Phase	ClinicalTrial.gov identifier (NCT number)	References
Tazepelumab (MEDI9929)	AD	Moderate-severe	2a	NCT02525094	⁶⁰
	Asthma	Uncontrolled, severe	2	NCT02054130	⁸⁴
			3 (ongoing)	NCT03347279, NCT03406078	NA
CSJ 117	Asthma	Mild	1	NCT03138811	NA

NA, nothing available.

evidence of type 2 inflammation and have non-Th2 (non-type 2) inflammation associated with IFN- γ or IL-17 derived from Th1 cells, Th17 cells, or ILC3s.⁶¹ These different inflammation patterns cause heterogeneity of clinical phenotypes of asthma.

TSLP polymorphism in asthma

Genome-wide association studies (GWASs) of asthma identified a single nucleotide polymorphism in the TSLP locus (rs1837253) that is associated with an increased risk of asthma in North American populations.⁶² Similarly, a GWAS study of Japanese asthmatic population indicated that the SNP of the TSLP locus (rs1837253) is associated with susceptibility to adult asthma.⁶³ This locus is located 5.7 kb upstream of the transcription initiation site of TSLP and was related to airway hyperresponsiveness in a candidate gene approach study.⁶⁴

The role of TSLP in the pathogenesis of asthma

An early study utilizing *in situ* hybridization and immunohistochemistry demonstrated that the number of TSLP mRNA-expressing cells within the bronchial epithelium and submucosa

is significantly increased in patients with asthma compared to normal control subjects.⁶⁵ In addition, bronchial epithelial, endothelial, and immune cells were shown to express TSLP.⁶⁵ TSLP expression levels are also significantly increased in airway epithelium and lamina propria of asthmatic patients, especially those with severe asthma, in spite of high-dose inhaled corticosteroid therapy.⁶⁶ Furthermore, TSLP concentrations in BAL fluids are significantly elevated in asthmatic patients.⁶⁷ These studies thus suggest that expression levels of TSLP are correlated with the severity of airflow obstruction independent of corticosteroid therapy.

The effect of TSLP on acquired immune cells in asthma

Mouse models of OVA-induced allergic airway inflammation show increased TSLP expression in lungs. In addition, TSLPR-deficient mice show significant suppression of OVA-induced type 2 inflammation.¹³ Mice overexpressing lung-specific TSLP (SPC-TSLP mice) spontaneously develop type 2 airway inflammation associated with infiltration of Th2 cells and increased serum IgE levels.¹³ Mechanistically, TSLP induces expression of OX40L by dendritic cells, which triggers Th2 cell polarization⁶⁸ and the proliferation of and IL-4 expression by CD4⁺ T cells.^{25,69,70}

The role of TSLP on innate immunity and cell populations in asthma (Fig. 2)

ILC2s play an important role in type 2 inflammation in asthma, especially eosinophilic asthma, severe asthma, and virus-induced asthma exacerbation.⁷¹ Although TSLP does not induce type 2 cytokine production by lung ILC2s, TSLP supports survival of ILC2s, along with IL-2 and IL-7.⁷² In contrast, IL-33 strongly induces proliferation and type 2 cytokine production by ILC2s. Interestingly, the combination of TSLP with IL-33 has a synergistic effect on proliferation and type 2 cytokine production of both mouse and human ILC2s.^{73,74} Furthermore, TSLP activates the anti-apoptotic

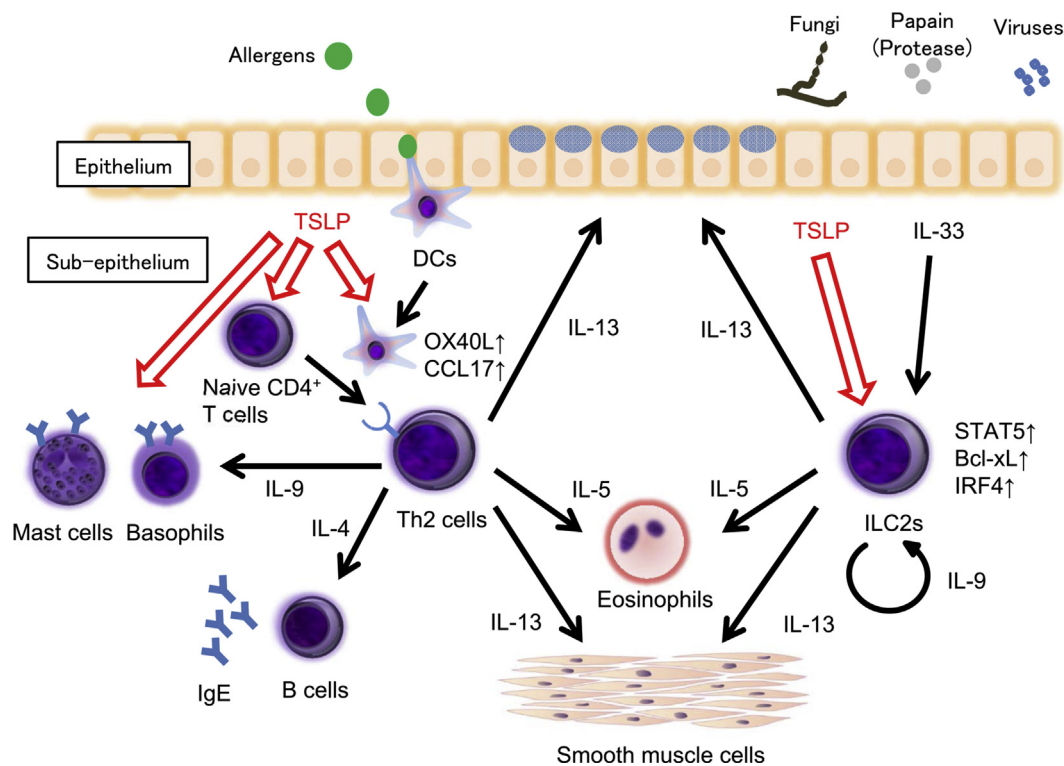


Fig. 2. The role of TSLP in asthma pathogenesis. TSLP stimulates DCs, CD4⁺ T cells, mast cells, and basophils, which promote allergen-induced Th2 cell-mediated immune responses in airways. Furthermore, TSLP and IL-33 activate ILC2s, enhancing ILC2-mediated immune responses and corticosteroid resistance.

molecule Bcl-xL via the STAT5 pathway in ILC2s, which induces resistance to corticosteroids.⁷⁵ TSLP also induces corticosteroid resistance in human ILC2s via STAT5 and MAP kinase,⁷⁴ with the number of ILC2s significantly increased in the sputum of patients with severe asthma.⁷⁶ The TSLP-ILC2 pathway thus appears involved in the pathophysiology of corticosteroid-resistant severe asthma. Many other immune cells also express TSLPR, and mast cells, eosinophils, and NKT cells all respond to TSLP.⁷⁷ For example, TSLP, IL-1 β , and TNF α synergistically activate human mast cells and induce production of type 2 cytokines like IL-5, IL-6, and IL-13.³⁰

Role of TSLP in respiratory viral infection-induced asthma exacerbation

Respiratory viral infections are a major cause of asthma exacerbation, and double-strand (ds) RNA, an indicator of viral infection, increases TSLP expression in bronchial epithelial cells.⁷⁸ Importantly, bronchial epithelial cells from patients with asthma exhibit higher levels of TSLP in response to dsRNA than bronchial epithelial cells from control subjects.⁷⁹ In general, respiratory viruses, such as rhinovirus, respiratory syncytial (RS) virus, and influenza virus, increase the production of epithelial cell-derived cytokines, like IL-33, IL-25, and TSLP. In addition, RS infections induce ILC2, which produces IL-13 and causes mucin overexpression. In this model system, an anti-TSLP antibody or the lack of TSLP in TSLP receptor-deficient mice reduces the number of IL-13⁺ ILC2s and reduces mucin overexpression.⁸⁰

Anti-TSLP antibody therapy for asthma

Tezepelumab has completed proof-of-concept and phase 2 randomized, double-blinded, placebo-controlled studies for evaluation of its efficacy and safety in subjects with asthma. In a proof-of-concept study (NCT01405963), 700 mg of tezepelumab or a placebo was intravenously administered every 4 weeks to 31 patients with mild allergic asthma.⁸¹ Allergen inhalation challenges

were performed two weeks before the first dose of tezepelumab, and on days 42 and 84 of treatment. The primary end point was the %decrease in FEV₁ during the late asthmatic response after allergen challenge and was lower in the tezepelumab-treated group by 34–46% on days 42 and 84 ($P = 0.09$ and 0.02 , respectively), compared to the placebo group. Interestingly, early asthmatic responses were also decreased in the tezepelumab group by 27–31% ($P = 0.05$ – 0.06), suggesting that tezepelumab suppresses TSLP to activate basophils and/or mast cells.⁸² Although there were no significant changes in FEV₁ levels possibly due to relatively good baseline pulmonary function, airway responsiveness to methacholine was significantly improved. Treatment with tezepelumab decreased eosinophil counts in peripheral blood/sputum, Th2/Th1 cell ratios in peripheral blood, and FeNO, suggesting that tezepelumab attenuates late asthmatic responses through effects on Th2 cells/ILC2s. There were no changes in CD4⁺CD25⁺CD127^{low}Foxp3⁺ regulatory T cell frequency.⁸³

In a phase 2 study (NCT02054130), 584 patients with uncontrolled asthma who had at least two asthma exacerbations in the previous year and Asthma Control Questionnaire (ACQ)-6 scores of at least 1.5 despite combined treatments of medium-to-high doses of ICS with long-acting β_2 agonists, were randomized 1:1:1:1 for treatment with 70 mg of subcutaneous tezepelumab every 4 weeks (low-dose), 210 mg tezepelumab every 4 weeks (medium-dose), 280 mg tezepelumab every 2 weeks (high-dose), or a placebo.⁸⁴ The primary endpoint was the asthma exacerbation rate during 52 week-study period, which was 0.26, 0.19, and 0.22 in the low-dose, medium-dose, and high-dose groups, respectively, compared to 0.67 in the placebo group. FEV₁ levels improved in the tezepelumab group by 0.11–0.15 L more than in placebo group and ACQ-6 scores were decreased. Blood eosinophil counts, FeNO, and total serum IgE levels were also decreased, supporting the hypothesis that the anti-TSLP antibody inhibits type 2 responses. Importantly, suppression of asthma exacerbation by tezepelumab was observed irrespective of peripheral blood eosinophil count or FeNO (Fig. 3), in contrast to previous reports showing that an anti-IL-4 receptor- α antibody and

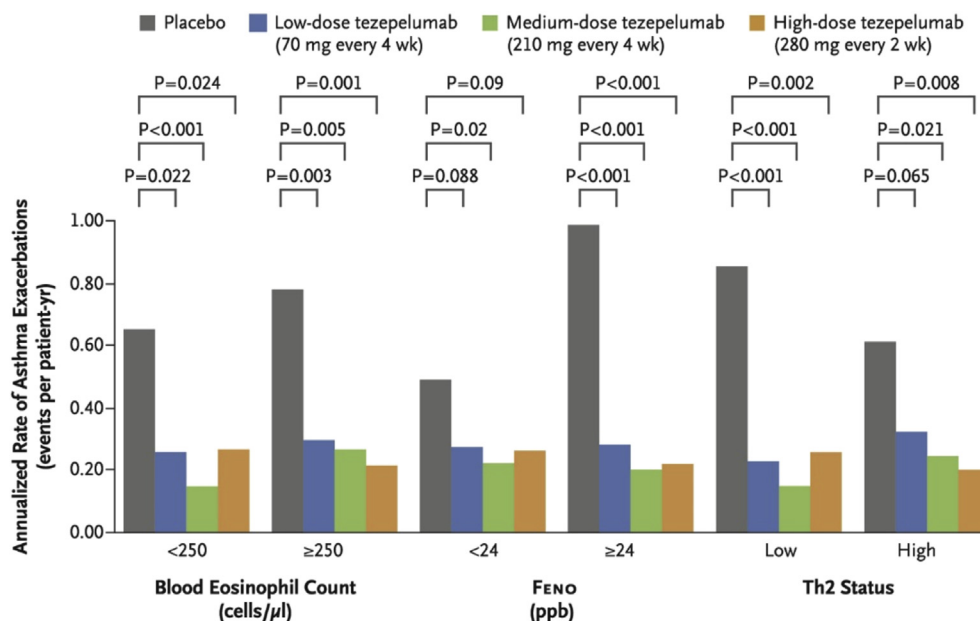


Fig. 3. Annualized rate of asthma exacerbation at week 52 based on baseline biomarker status in patients treated with tezepelumab or a placebo. Patients with uncontrolled asthma were randomized 1:1:1:1 for treatment with 70 mg subcutaneous tezepelumab every 4 weeks (low-dose, $n = 145$), 210 mg tezepelumab every 4 weeks (medium-dose, $n = 145$), 280 mg tezepelumab every 2 weeks (high-dose, $n = 146$), or a placebo ($n = 148$). Annualized rates of asthma exacerbation at week 52 was compared based on peripheral blood eosinophil counts, FeNO, and Th2 status. A Th2 high status was defined as an IgE level of more than 100 IU/mL and a blood eosinophil count of 140 cells/μL. Reproduced from Ref. 84 with permission.

anti-IL-5/IL-5 receptor- α antibodies exhibit limited efficacy in non-type 2 asthma.

Currently, two phase 3 trials for tezepelumab in patients with uncontrolled, severe asthma are underway. The NAVIGATOR trial (NCT03347279) will examine > 1000 patients with uncontrolled asthma under medium to high doses of ICS and other controller(s) randomized to either subcutaneous tezepelumab or placebo, and the primary endpoint is the annual asthma exacerbation rate. The SOURCE trial (NCT03406078) will examine whether tezepelumab treatment can reduce the required daily doses of oral corticosteroids without loss of asthmatic symptom control.

CSJ 117 is a once-daily inhaled formulation of a monoclonal antibody fragment (Fab) targeting TSLP. A 12-week phase 1 trial (NCT03138811) in patients with mild asthma has just been completed, but no data are available yet.

Conclusion

TSLP is considered a master regulator for the transition to type 2 inflammation at the barrier surface of the skin, airways/lungs, and gut. This hypothesis is supported by genetic, experimental, and clinical evidence and has now been confirmed by the effects of anti-TSLP antibody in patients with allergic diseases. However, the clinical efficacy of thus anti-TSLP antibody in patients with established allergic diseases cannot solely be explained by effects on dendritic cells and Th2 cells and likely involves additional biological functions of the TSLP-TSLPR pathway in other immune cells, such as basophils and non-immune cells such as neurons. Further study is required to extend the efficacy and the range of anti-TSLP antibodies in allergic diseases.

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Conflict of interest

KK serves as a consultant to Chugai, A*Star, Maruho and received a research grant from Japan Tobacco, Mitsubishi Tanabe, Torii Pharmaceutical, Pola Pharma, Eisai, Taiho Pharmaceutical, Ono Pharmaceutical, Kyowa Kirin, Sanofi. KA received lecture fees from AstraZeneca and received a research grant from Novartis Pharma, Sanofi, Kyorin Pharma. The rest of the authors have no conflict of interest.

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