



BASIC SCIENCE ARTICLE

Anti-thymic stromal lymphopoietin antibody suppresses airway remodeling in asthma through reduction of MMP and CTGF

Sheng-Chieh Lin^{1,2,3}, Hsiu-Chu Chou⁴, Chung-Ming Chen^{2,5} and Bor-Luen Chiang³

BACKGROUND: Thymic stromal lymphopoietin (TSLP) mediates immune reaction in patients with asthma. Matrix metalloproteinase (MMP), connective tissue growth factor (CTGF), and transforming growth factor- β (TGF- β) are inflammatory mediators whose responses to the anti-TSLP antibody are unknown. This study examined the effect of an anti-TSLP antibody on MMP, CTGF, TGF- β , and airway structural changes in airway remodeling in asthma.

METHODS: Mice were randomly divided into phosphate-buffered-saline-challenged (PBS), ovalbumin-challenged (OVA), and ovalbumin-challenged with anti-TSLP antibody (OVA + anti-TSLP) groups. Airway responsiveness and serum ovalbumin-specific immunoglobulin E were measured. Differential cell counts and MMP-2 and MMP-9 were evaluated in bronchoalveolar lavage fluid (BALF). Airway structural changes were quantified using morphometric analysis and presentation by immunohistochemistry staining. Lung CTGF, TGF- β , and TSLP were analyzed using western blot.

RESULTS: Airway responsiveness was significantly lower in OVA + anti-TSLP and PBS groups than in OVA group. Airway structural changes exhibited less smooth muscle thickness in OVA + anti-TSLP and PBS groups than in OVA group. MMP-2 and MMP-9 in BALF and CTGF, TGF- β , and TSLP in lungs significantly decreased in OVA + anti-TSLP and PBS groups compared with OVA group.

CONCLUSION: Anti-TSLP antibody exerts the preventive effect of decreasing airway structural changes through reduction of MMP, TGF- β , and CTGF in airway remodeling of asthma.

Pediatric Research (2019) 86:181–187; <https://doi.org/10.1038/s41390-018-0239-x>

INTRODUCTION

Asthma is an inflammatory airway disease characterized by wheezing breathing sounds, airflow obstruction, and the presence of variable immune cells. Increased airway smooth muscle mass since childhood (preschool age) might be a crucial factor in subsequent asthma development.¹ Although substantial progress has been made in understanding asthma, the precise cause remains unknown. The complex interrelationships between genetic, environmental, pharmacologic, and immunologic factors in asthma require further investigation.² In asthma, many immune cells, including dendritic cells, T cells, B cells, eosinophils, basophils, and mast cells, infiltrate the bronchial submucosa and cause a series of immune reactions.³ The inflammatory cells in the airway exhibit altered repair responses, including the secretion of cytokine and growth factors that induce airway structural changes termed airway remodeling.^{4,5} Airway structural changes include an elevated number of inflammatory cells, goblet cell metaplasia, hypertrophy of submucosal glands, hyperplasia of airway smooth muscle, deposition of collagen and fibronectin in the subepithelial basement membrane, and abnormal extracellular matrix deposition.^{4,6} Matrix metalloproteinases (MMPs) belong to a family of extracellular proteases that are responsible for the degradation of the extracellular matrix during tissue remodeling.⁷ Expression and

activity of MMPs are regulated through tissue inhibitors of metalloproteinases (TIMPs) and CD147.⁸ CD147 is expressed on the surface of various cells, including epithelial, endothelial, immune, hematopoietic, and smooth muscle cells, and induces MMP production.⁹ MMP-2 and MMP-9 affect the extracellular matrices of gelatins, collagens, and elastin, and also interact with the transforming growth factor- β (TGF- β).⁷ Expression of MMP-2 and MMP-9 in bronchoalveolar lavage fluid (BALF) is high during chronic airway inflammation.¹⁰ Connective tissue growth factor (CTGF), which is classified in the CTGF/Cyr61/Nov family, is another TGF- β target gene.¹¹ Some of the molecules that interact with CTGF are growth factors, cytokines, receptors, and matrix proteins.¹² CTGF has been described as a downstream of the profibrotic TGF- β effect that enables cell adhesion, migration, hypertrophy, proliferation, and extracellular matrix synthesis.^{11,13} Expressions of MMP and CTGF are upregulated in airway remodeling and play a crucial role in asthma pathogenesis.¹⁴

Several studies have focused on thymic stromal lymphopoietin (TSLP)—an epithelial-derived cytokine that was first identified in a culture supernatant of murine thymic stromal cells.¹⁵ TSLP induces an immune response toward a Th2 phenotype and may orchestrate tissue inflammation through Th1 and Th17 cytokines.^{16,17} TSLP-influenced pulmonary Treg increases protein

¹Department of Pediatrics, Shuang Ho Hospital, Taipei Medical University, Taipei, Taiwan; ²Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan; ³Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan; ⁴Department of Anatomy and Cell Biology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan and ⁵Department of Pediatrics, Taipei Medical University Hospital, Taipei, Taiwan
Correspondence: C-M. Chen (cmchen@tmu.edu.tw) or B-L. Chiang (gicmbor@ntu.edu.tw)
These authors contributed equally: Chung-Ming Chen and Bor-Luen Chiang.

Received: 25 August 2018 Accepted: 2 November 2018
Published online: 21 November 2018

expression in airway BALFs and provides tolerogenic immunity to patients with asthma.¹⁸ Environmental stimuli, allergen, and viruses may stimulate bronchial epithelium in asthma to increase TSLP.¹⁹ TSLP expression in asthma was upregulated through the bronchial epithelium and airway smooth muscle.²⁰ Expressions of TSLP and TARC/CCL17 were correlated with airway obstruction in asthma.²¹ TSLP and TSLP receptor (TSLPR) heterocomplex expression regulate innate-adaptive immunity in pediatric asthma.²² In addition, TSLP-transgenic mice developed spontaneous inflammation with infiltration of goblet cell hyperplasia, leukocytes, and subepithelial fibrosis in the lung.²³ TSLP seems to play a key role in inflammation in asthma pathogenesis.² The present study examined the effect of said anti-TSLP antibody on airway structural changes in airway remodeling, and determined the influence of an anti-TSLP antibody on MMP, TGF- β , and CTGF, all of which are involved in the airway remodeling of asthma.

METHODS

Murine model of asthma

The Institutional Animal Care and Use Committee of Taipei Medical University approved the protocol of the present study. Eight-week-old female BALB/c mice fed in a pathogen-free facility were purchased from BioLASCOTaiwan Co., Ltd. These mice received standard animal care under the supervision of Institutional Animal Care and Use Committee of Taipei Medical University. They were kept in a facility maintained at approximately 25 °C and provided with pelleted food and water ad libitum throughout the experiment. Seventeen mice were randomly divided into a phosphate-buffered-saline-challenged (PBS) group (negative control group, $n = 6$), ovalbumin-challenged (OVA) group (positive control group, $n = 6$), and ovalbumin-challenged with anti-TSLP (OVA + anti-TSLP) group (therapeutic group, $n = 5$).

The mice in the PBS group were given an intraperitoneal injection of 2 mg of aluminum hydroxide (Imject Alum; Pierce, Rockford, IL) in 0.2 mL of phosphate-buffered saline on day 1 and an intraperitoneal injection with 4 mg of aluminum hydroxide in 0.2 mL of phosphate-buffered saline on days 15, 22, and 29. These mice were then intranasally challenged with 50 μ L of aerosolized phosphate-buffered saline solution on days 36, 37, 38, 39, 40, 41, 42, and 43. The mice in the OVA group were sensitized using an intraperitoneal injection of 20 μ g of Grade V chicken egg ovalbumin (Sigma Chemical Co., St. Louis, MO) and 2 mg of aluminum hydroxide in 0.2 mL of phosphate-buffered saline on day 1, and an intraperitoneal injection of 50 μ g of Grade V chicken egg ovalbumin and 4 mg of aluminum hydroxide in 0.2 mL of phosphate-buffered

saline 60 minutes before Grade V chicken egg ovalbumin sensitization, and then intranasally challenged with 100 μ g of Grade V chicken egg ovalbumin in a total volume of 40 μ L of phosphate-buffered saline on days 36, 37, 38, 39, 40, 41, 42, and 43. Sixty minutes before being intranasally challenged with Grade V chicken egg ovalbumin, these mice were anesthetized using 2% isoflurane inhalant (Halocarbon Laboratories, River Edge, NJ), and then 20 μ g of isotype control IgG2 antibody (MAB006; R&D systems) in 40 μ L of phosphate-buffered saline was intranasally delivered dropwise into the nares by using a Pipetman pipette (model P200, Gilson) while the mouse was held in an erect position. The mice in the OVA + anti-TSLP group were sensitized using an intraperitoneal injection of 20 μ g of Grade V chicken egg ovalbumin and 2 mg of aluminum hydroxide in 0.2 mL of phosphate-buffered saline on day 1; they were subsequently sensitized using an intraperitoneal injection of 50 μ g of Grade V chicken egg ovalbumin and 4 mg of aluminum hydroxide in 0.2 mL of phosphate-buffered saline on days 15, 22, and 29. These mice were intraperitoneally injected with 20 μ g of anti-TSLP monoclonal antibody (MAB555; R&D systems) in 0.2 mL of phosphate-buffered saline 60 minutes before Grade V chicken egg ovalbumin sensitization. They were then intranasally challenged with 100 μ g Grade V chicken egg ovalbumin in 40 μ L of phosphate-buffered saline on days 36, 37, 38, 39, 40, 41, 42, and 43. Sixty minutes before being intranasally challenged with Grade V chicken egg ovalbumin, these mice were anesthetized using 2% isoflurane inhalant, and 20 μ g of anti-TSLP monoclonal antibody in 40 μ L of phosphate-buffered saline was intranasally delivered dropwise into the nares by using a Pipetman pipette while the mouse was held in an erect position (Fig. 1).

Measurement of airway responsiveness through plethysmography
Airway responsiveness was measured using barometric whole-body plethysmography to calculate enhanced pause (Penh; Buxco Electronics Inc., Sharon, CT) on day 44. A technician who was blinded to the experimental groups and study protocol operated the plethysmograph. The mice were first exposed to phosphate-buffered saline inhalation and subsequently given increasing methacholine doses (Sigma, St Louis, MO) of 6.25, 12.5, 25, and 50 mg/mL. Each nebulization required 3 min, and data were recorded at 3 min after nebulization. To enable airway Penh to return to its baseline level, every aerosol was separated with a 15-min recovery period. The mean of the first 3-min records after exposure to each methacholine or placebo are included in this report. The Penh results are expressed as absolute values.

Evaluation of serum ovalbumin-specific IgE

The mice received intraperitoneal injections of pentobarbital (100 mg/kg, Sigma, St Louis, MO) after airway responsiveness measurement on day 45. After the mice were anaesthetized,

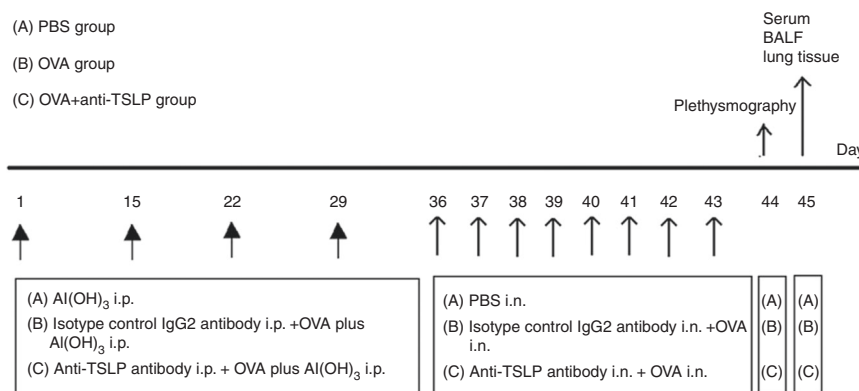


Fig. 1 Murine model of asthma (A) PBS group (B) OVA group, and (C) OVA + anti-TSLP group

serum was collected through cardiac puncture and stored at -20°C for measurement of ovalbumin-specific IgE. Serum ovalbumin-specific IgE levels were measured using the enzyme-linked immunosorbent assay (ELISA) method (BioLegend, San Diego, CA).

Evaluation of BALF

Immediately after cardiac puncture, a tracheostomy tube was inserted through the trachea to the lungs. The tube was instilled with 1 mL of ice-cold saline, which was washed in and out of the lungs three times and then collected. This washing procedure was repeated thrice and then the total volume of the BALF was recorded. Differential cell counts of BALF with standard morphologic criteria were checked using cytocentrifuge preparations (Cytospin 3; Shandon Scientific, Cheshire, UK) stained with Liu's stain (Tonyar, Diagnostic Inc., Taiwan). The levels of MMP-2 and MMP-9 in the BALF were evaluated using the ELISA method (Cloud-Clone Corp., Houston) in accordance with the manufacturer's instructions.

Evaluation of morphometric analysis and immunohistochemistry staining

Airway structural changes were evaluated by a pathologist (Chou HC) who was blinded to the experimental groups. Lung sections of $7\mu\text{m}$ were embedded in paraffin and stained with hematoxylin and eosin. The pathologist analyzed three bronchial segments measuring $150\text{--}350\mu\text{m}$ in luminal diameter per mouse to evaluate the thickness of the epithelium and smooth muscle layers. Peribronchial inflammation was quantified by counting the number of inflammatory cells surrounding airways, and the results were normalized for airway size by dividing them by square of the perimeter of the basement membrane.¹⁴ In order to measure the number of goblet cells, lung sections were stained with periodic acid schiff (PAS). Immunohistochemistry staining of α -actin for smooth muscle (Alpha-SMA) from lung sections were performed. Immunohistochemistry staining of CTGF, TGF- β from lung sections were also performed.

Evaluation of western blot analysis in lung tissue

To evaluate the expressions of CTGF, TGF- β , and TSLP in lung tissue in all three groups of mice, western blot analysis was performed. Lung tissue samples were homogenized in ice-cold buffer containing 50 mM of Tris-HCl (pH 7.5), 1 mM of ethylenediaminetetraacetic acid, 1 mM of egtazic acid, and a protease inhibitor cocktail (complete minitables, Roche, Mannheim, Germany). Proteins (30 μg) were dissolved in 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis under reducing conditions and electroblotted to a polyvinylidene difluoride membrane (ImmobilonP, Millipore, Bedford, MA). After being blocked with 5% nonfat dry milk, the membranes were incubated with anti-CTGF (1:2000, Abcam, Cambridge, UK), anti-TGF- β (1:2000, Santa Cruz biotechnology, Dallas, Texas), anti-TSLP (1:2000, Abcam, Cambridge, UK), or anti- β -actin (1:5000, Santa Cruz biotechnology, Dallas, Texas) and then incubated with horseradish peroxidase-conjugated goat antimouse IgG (Pierce Biotechnology, Rockford). Protein bands were detected using SuperSignal Substrate from Pierce Biotechnology. Densitometric analysis was performed to measure the intensity of CTGF, TGF- β , TSLP, and β -actin bands by using AIDA software.

Statistical analysis

The groups were compared using the case-control method and Student's *t* test. We evaluated the levels of IgE, MMP, CTGF, TGF- β , and TSLP in the OVA + anti-TSLP and PBS groups for comparison with the OVA group. Comparisons among the three groups were made based on a one-way analysis of variance followed by Bonferroni's analysis. The results are presented as means \pm standard deviations (SDs). Differences were considered significant at $P < 0.05$. All data analyses were performed using STATA 12.

RESULTS

Airway responsiveness (Penh)

The airway responses in enhanced pause (Penh) in the PBS, OVA, and OVA + anti-TSLP groups at various methacholine concentrations are shown in Fig. 2. Penh was significantly lower in the PBS and OVA + anti-TSLP groups than in the OVA group at challenged methacholine concentrations of 12.5, 25, and 50 mg/mL. Airway resistance decreased more in the PBS group than in the OVA group, and also decreased in the OVA + anti-TSLP group.

Serum ovalbumin-specific IgE

Ovalbumin-specific IgE levels in serum were significantly (14.5-fold) lower in the PBS group than in the OVA group (Fig. 3). Ovalbumin-specific IgE levels were nonsignificantly (1.3-fold) lower in the OVA + anti-TSLP group than in the OVA group.

Differential Cell count in BALF

The bronchoalveolar lavage differential cells in the three groups are described as follows: the PBS group showed 0% eosinophils, $40.00\% \pm 27.22\%$ neutrophils, $60.00\% \pm 40.37\%$ lymphocytes, and 0% macrophages. The OVA group showed $1.82\% \pm 1.24\%$ eosinophils, $93.42\% \pm 3.04\%$ neutrophils, $4.16\% \pm 1.53\%$ lymphocytes, and $0.60\% \pm 0.44\%$ macrophages. The anti-TSLP group showed 0% eosinophils, $71.16\% \pm 40.00\%$ neutrophils, $15.76\% \pm 19.34\%$ lymphocytes, and $13.08\% \pm 20.70\%$ macrophages (Table 1).

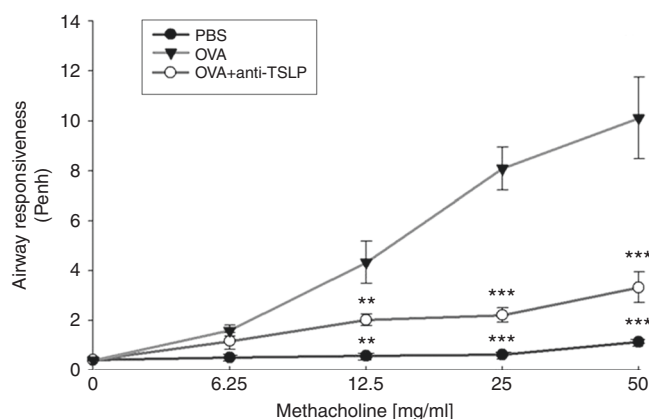


Fig. 2 Enhanced pause (Penh) as a function of methacholine concentration. Values are given as means \pm SDs. Black circle, Phosphate-buffered-saline-challenged mice (PBS group); Black downward triangle, ovalbumin-challenged mice (OVA group); White circle, ovalbumin-challenged with anti-TSLP antibody intervention mice (OVA + anti-TSLP group). Penh was significantly lower in the OVA + anti-TSLP and PBS groups than in the OVA group at methacholine concentrations of 12.5, 25, and 50 mg/ml (** $P < 0.01$, *** $P < 0.001$ vs. OVA group)

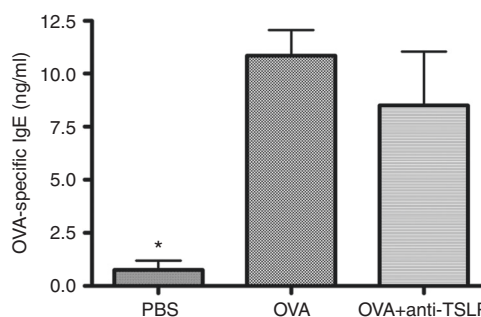


Fig. 3 Mouse serum levels of ovalbumin-specific IgE. Serum ovalbumin-specific IgE levels decreased significantly in the PBS group compared with the OVA group (* $P < 0.05$ vs. OVA group)

Table 1. Bronchoalveolar lavage differential cell counts

Group	Eosinophils (%)	Neutrophils (%)	Lymphocytes (%)	Macrophages (%)
PBS	0.00 ± 0.00*	40.00 ± 27.22**	60.00 ± 40.37*	0.00 ± 0.00
OVA	1.82 ± 1.24	93.42 ± 3.04	4.16 ± 1.53	0.60 ± 0.44
OVA + anti-TSLP	0.00 ± 0.00*	71.16 ± 40.00	15.76 ± 19.34	13.08 ± 20.70

Differential cells of BALF in all three groups were counted on cytocentrifuge preparations in accordance with standard morphologic criteria. The results are expressed as percentages in the total number of cells. Values are given as means ± SDs

* $P < 0.05$; ** $P < 0.01$ vs. OVA group

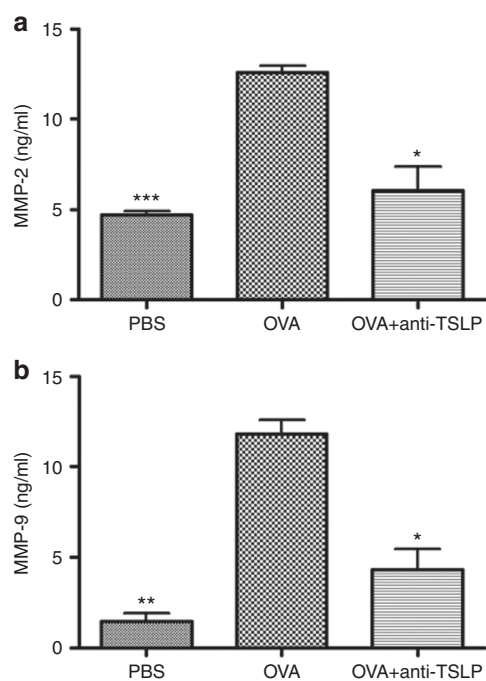


Fig. 4 Concentrations of **a** MMP-2 and **b** MMP-9 in BALF. MMP-2 and MMP-9 decreased significantly in the OVA + anti-TSLP and PBS groups compared with the OVA group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. OVA group)

The differential cell count of BALF revealed a significantly decreased percentage of eosinophils, neutrophils, and lymphocytes in the PBS group compared with the OVA group, as well as a decreased percentage of eosinophils in the OVA + anti-TSLP group compared with the OVA group.

Concentrations of MMP-2 and MMP-9 in BALF

The MMP-2 concentrations in the PBS ($P < 0.001$) and OVA + anti-TSLP ($P < 0.05$) groups were significantly lower than that in the OVA group (Fig. 4a). Furthermore, the MMP-9 concentrations in the PBS ($P < 0.01$) and OVA + anti-TSLP ($P < 0.05$) groups were significantly lower than that in the OVA group (Fig. 4b). Thus, MMP-2 and MMP-9 concentrations decreased in the ovalbumin-sensitized mice with anti-TSLP antibody intervention.

Lung histology, immunohistochemistry staining and morphometric analysis

The lungs of the OVA group exhibited hypertrophy of smooth muscle, goblet cells and a high number of inflammatory cells concentrated near the airway and in the perivascular area (Fig. 5a–c). The thickness of the smooth muscle layer and the numbers of goblet cells and total inflammatory cells were significantly lower in the PBS group than in the OVA group

(Fig. 5d). Furthermore, the thickness of the smooth muscle layer significantly decreased in the OVA + anti-TSLP group compared with the OVA group (Fig. 5d). Thus, the thickness of the smooth muscle layer decreased in the ovalbumin-sensitized mice with anti-TSLP antibody intervention. In Fig. 6a, b, the IHC analyses of CTGF and TGF- β in lung were illustrated.

Western blot analysis of CTGF, TGF- β , and TSLP

The western blot analysis was illustrated in Fig. 6c. CTGF protein expression in lung tissue was significantly lower in the PBS and OVA + anti-TSLP groups than in the OVA group (Fig. 6d). TGF- β protein expression in lung tissue was significantly lower in the PBS and OVA + anti-TSLP groups than in the OVA group (Fig. 6d). TSLP protein expression in lung tissue was lower in the PBS and OVA + anti-TSLP groups than in the OVA group (Fig. 6d). Thus, the expressions of CTGF, TGF- β , and TSLP decreased in the ovalbumin-sensitized mice with anti-TSLP antibody intervention.

DISCUSSION

TSLP is an epithelial cell-derived cytokine that may be expressed in the lungs and may regulate signals via a TSLPR complex—a heterodimer of the interleukin (IL)-7 receptor α chain and TSLPR chain.²¹ After stimulation by allergens, viruses, irritants, pollutants, endotoxins, and CpG DNA, airway epithelial cells may secrete TSLP.²² TSLP was overexpressed and inflammation was induced in the lungs of mice with asthma.²³ Humans with asthma also express substantially increased TSLP in bronchial epithelial cells in the lungs.²¹ Furthermore, a higher level of TSLP was noted in asthmatic children than in healthy children.² Overexpression of TSLP after exposure to stimuli was found in mouse and human airway epithelial cells, and these cells interacted with proinflammatory mediators, including tumor necrosis factor alpha, IL-1, and selected toll-like receptor (TLR) agonists (TLR2, TLR8, and TLR9 ligands).²⁴ TSLP can activate dendritic cells, mast cells, natural killer T cells, and eosinophils and interact with cytokines and inflammatory mediators to influence the airway smooth muscle of patients with asthma.²² Studies have reported that epithelial cells, fibroblasts, airway smooth muscle cells, and mast cells in airways may overexpress TSLP in asthmatic disease and cause inflammation.^{19,20}

We used a murine model to investigate the anti-TSLP antibody effect on inflammation and smooth muscle in the airway remodeling of asthma. Lung inflammation with excessive bronchial smooth muscle remodeling in murine models occurred with ovalbumin-induced mice.²⁵ To evaluate the ability of a drug to suppress airway inflammation, airway remodeling, and airway hyperresponsiveness were applied in a murine model of asthma induced by administration of ovalbumin.¹² Airway hyperresponsiveness and airway obstruction are characteristics of asthma. TSLP expression presented in bronchial epithelial cells was correlated with forced expiratory volume in 1 sec in asthma.²¹ Penh differed significantly among the three groups in the present study; Penh was significantly lower in the OVA + anti-TSLP and PBS groups than in the OVA group. The anti-TSLP antibody appears to

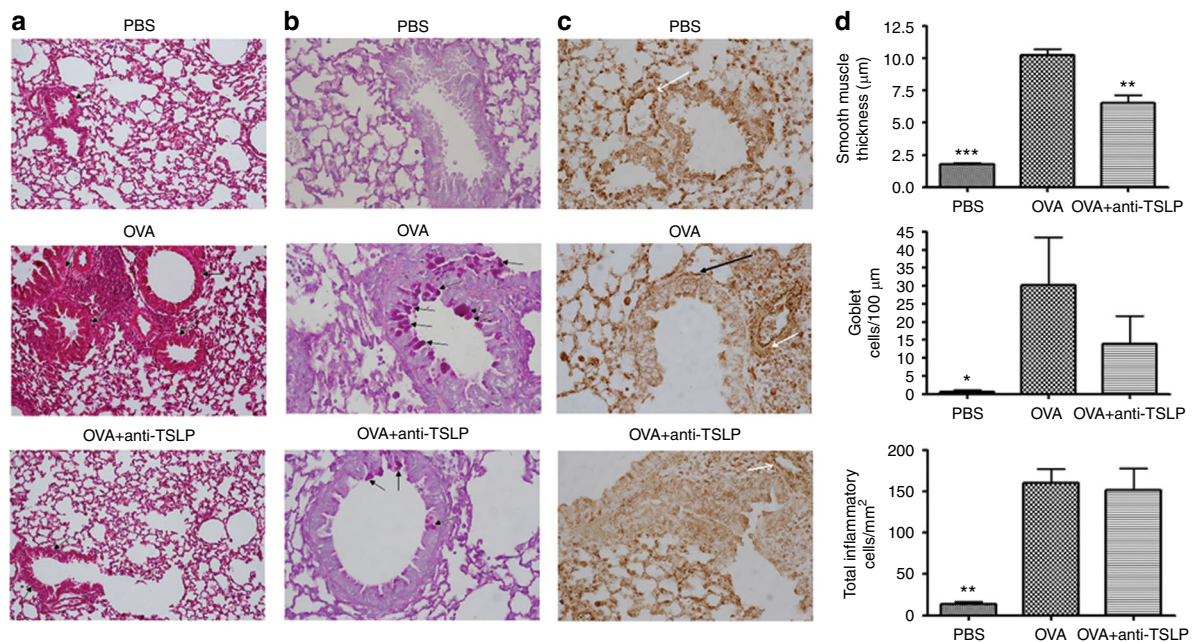


Fig. 5 Representative photomicrographs **a** HE stain (100 \times , black arrow: smooth muscle) **b** PAS stain (400 \times , black arrow: goblet cell), **c** IHC staining of alpha-SMA, (400 \times , black arrow: alpha-SMA + smooth muscle layer of bronchiole; white arrow: alpha-SMA + smooth muscle layer of artery) **d** morphometric analysis of structural changes. Three bronchial segments measuring 150–350 μ m in luminal diameter per mouse were analyzed for thickness of the epithelium and smooth muscle layers and numbers of total inflammatory cells and goblet cells surrounding the airways. The thickness of the bronchial epithelium and smooth muscle layer and the numbers of total inflammatory cells and goblet cells decreased significantly in the PBS group compared with the OVA group. The thickness of the bronchial epithelium and smooth muscle layer decreased significantly in the OVA + anti-TSLP compared with the OVA group (* p < 0.05, ** p < 0.01, *** p < 0.001 vs. OVA group)

improve airway hyperresponsiveness in the airway remodeling model of asthma.

In this study, the OVA group exhibited a significantly higher serum ovalbumin-specific IgE level compared with the PBS group. The OVA + anti-TSLP group exhibited a decreased serum ovalbumin-specific IgE level, although the difference was non-significant. These results were consistent with those of Gauvreau et al., who reported that anti-TSLP antibody had no effect on levels of serum total IgE in asthma.²⁶

In our study, the PBS and OVA + anti-TSLP groups had 0% eosinophils in BALF. The levels of TGF- β , CTGF, MMP-2, and MMP-9 differed significantly among the three groups. The PBS and OVA + anti-TSLP groups had significantly lower levels of TGF- β , CTGF, MMP-2, and MMP-9 than did the OVA group. Moreover, smooth muscle thickness in the airway differed significantly among the three groups. The PBS and OVA + anti-TSLP groups had significantly thinner smooth muscle than did the OVA group. TSLP is an essential and initial factor in airway inflammation in asthma.²³ TSLP is associated with bronchial asthma and TSLP genes are associated with allergic inflammation mechanisms, including eosinophil levels.¹⁶ In another mice study, TSLP exhibited the function of promoting T-cell and B-cell development and activation of mast cells and eosinophils.²⁷ Eosinophils produce proinflammatory cytokines, including IL-5, IL-6, IL-11, and IL-17, all of which play a role in airway remodeling.²⁸ In inflammatory airways, eosinophils infiltrate and also increase the number of eosinophil-derived TGF- β .²⁹ TGF- β 1 plays a crucial role in the development of airway remodeling in murine models of asthma induced through ovalbumin administration.^{30,31} TGF- β induces proliferation and differentiation of fibroblast cells into myofibroblasts and protein synthesis during the development of sub-epithelial fibrosis.³² TGF- β also induces proliferation and survival of extracellular matrix secretion in airway smooth muscle cells, which may cause increased thickness of airway tissues in airway remodeling of asthma.³² A decrease in TGF- β production prevents

the development of airway remodeling and possibly prevents therapeutic intervention during chronic asthma.³² CTGF in murine fibroblast cell lines was initially recognized as an immediate early gene that was activated with many cytokines, including TGF- β .¹¹ Interaction of CTGF with TGF- β causes activation of extracellular matrix deposition and remodeling; these factors together lead to tissue remodeling and changes in the organ structure.¹² TGF- β plays a crucial role in CTGF production, process, and action in various cells.¹¹ Inhibition of CTGF expression can prevent and reverse pathophysiologic tissue remodeling and the fibrosis process.¹² In addition, expression and activity of TGF- β and TGF- β receptors affect MMP and TIMP expression and activity in the vein wall.³³ TGF- β and growth factors also serve as inflammatory cytokines in MMP-9 secretion and production.³⁴ Knockdown of TGF- β 1 expression from cells affect MMP-9 gene expression.³⁵ Both MMP-2 and MMP-9 levels increase in BALF in OVA-challenged airway remodeling in asthmatic mice.¹⁴ We demonstrated the possible effect of an anti-TSLP antibody in preventing airway structural changes of smooth muscle thickness and decreasing the inflammatory mediators of TGF- β , CTGF, and MMP in airway remodeling of asthma.

Anti-TSLP antibodies decrease sputum and blood eosinophils and reduce allergen-induced bronchoconstriction in patients with allergen-induced asthma.²⁶ TSLP may induce corticosteroid resistance in patients with asthma through activation of natural helper cells,³⁶ and this may decrease corticosteroid efficiency and increase the risk of asthma exacerbation. In phase 2 clinical trials, human anti-TSLP antibodies have decreased the annualized rate of asthma attacks in patients with uncontrolled asthma who were already being treated with medium-to-high doses of inhaled glucocorticoids and long-acting β -agonists.^{22,37} Lung density decreased and then remained stable after anti-TSLP antibody therapy in a murine model of pulmonary fibrosis.²⁷ Until now, no clinical therapeutic interventions that may cure airway remodeling of asthma once it is established have been developed. Preventive

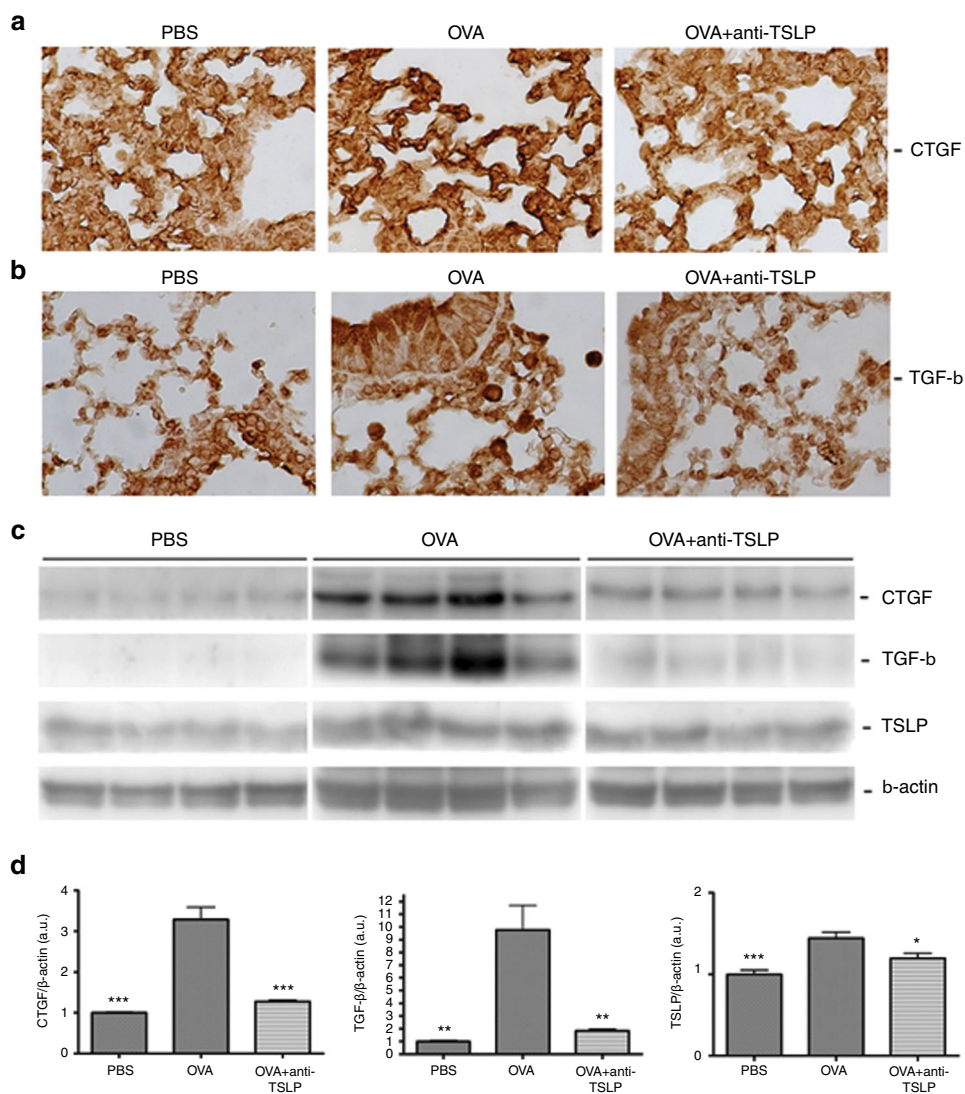


Fig. 6 Representative IHC staining, western blotting and scanning densitometry results **a** IHC staining of CTGF (400 \times) **b** IHC staining of TGF- β (400 \times). **c** Expressions of CTGF, TGF- β , and TSLP proteins. **d** Data were reported as the degree of change relative to the PBS group. Levels of CTGF, TGF- β , and TSLP expression were significantly lower in the OVA + anti-TSLP and PBS groups than in the OVA group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. OVA group)

interventions and treatment for airway remodeling in asthma are a priority and warrant further research.³⁸ One research group reported that IL-33 is a relatively steroid-resistant mediator that causes severe therapy-resistant asthma in patients and promotes airway remodeling.³ These findings suggest that IL-33 may be a crucial therapeutic target of airway remodeling in asthma; however, no clinical trials that support this have been conducted, and further evaluation is required.³ Thus, the anti-TSLP antibody is an effective alternative therapy for treating asthma, exerting positive effects in patients with uncontrolled asthma and corticosteroid resistance, and preventive effects on airway remodeling. Our experiment was performed using a small number of mice; despite this limitation, we demonstrated a significant effect on airway remodeling in asthma.

In conclusion, the present study demonstrated that the anti-TSLP antibody decreased airway hyperresponsiveness and suppressed airway remodeling in a murine model of asthma. This improvement in airway remodeling was accompanied by decreases in MMP-2 and MMP-9 in BALF and CTGF and TGF- β levels in lungs. The current findings suggest potential therapeutic

and preventive benefits of the anti-TSLP antibody for patients with asthma by reduction of MMP, TGF- β , and CTGF.

ACKNOWLEDGEMENTS

This work was supported by grants from Taipei Medical University (TMU106-AE1-B01).

ADDITIONAL INFORMATION

Competing interests: The authors declare no conflict of interest.

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REFERENCES

- O'Reilly, R. et al. Increased airway smooth muscle in preschool wheezers who have asthma at school age. *J. Allergy Clin. Immunol.* **131**, 1024–1032 (2013).
- Lin, S. C. et al. Upregulated thymic stromal lymphopoietin receptor expression in children with asthma. *Eur. J. Clin. Invest* **46**, 511–519 (2016).
- Saglani, S. et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J. Allergy Clin. Immunol.* **132**, 676–685 (2013).

4. Boulet, L. P. & Sterk, P. J. Airway remodeling: the future. *Eur. Respir. J.* **30**, 831–834 (2007).
5. Mauad, T., Bel, E. H. & Sterk, P. J. Asthma therapy and airway remodeling. *J. Allergy Clin. Immunol.* **120**, 997–1009 (2007).
6. Royce, S. G. et al. Intranasally administered serelaxin abrogates airway remodeling and attenuates airway hyperresponsiveness in allergic airways disease. *Clin. Exp. Allergy* **44**, 1399–1408 (2014).
7. Lu, P., Takai, K., Weaver, V. M. & Werb, Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb. Perspect. Biol.* **3**, a005058 (2011).
8. Agrawal, S. M., Silva, C., Wang, J., Tong, J. P. & Yong, V. W. A novel anti-EMMPRIN function-blocking antibody reduces T cell proliferation and neurotoxicity: relevance to multiple sclerosis. *J. Neuroinflamm.* **9**, 64 (2012).
9. Qu X., Wang C., Zhang J., Qie G., Zhou J. The Roles of CD147 and/or cyclophilin a in kidney diseases. *Mediators Inflamm.* **2014**:728673 (2014).
10. Rossi, H. S., Koho, N. M., Ilves, M., Rajamäki, M. M. & Mykkänen, A. K. Expression of extracellular matrix metalloproteinase inducer and matrix metalloproteinase-2 and -9 in horses with chronic airway inflammation. *Am. J. Vet. Res.* **78**, 1329–1337 (2017).
11. Moussad, E. E. & Brigstock, D. R. Connective tissue growth factor: what's in a name? *Mol. Genet Metab.* **71**, 276–292 (2000).
12. Lipson, K. E., Wong, C., Teng, Y. & Spong, S. CTGF is a central mediator of tissue remodeling and fibrosis and its inhibition can reverse the process of fibrosis. *Fibrogenes. Tissue Repair* **5**(Suppl 1), S24 (2012).
13. Wickert, L., Chatain, N., Kruschinsky, K. & Gressner, A. M. Glucocorticoids activate TGF-beta induced PAI-1 and CTGF expression in rat hepatocytes. *Comp. Hepatol.* **6**, 5 (2007).
14. Lin, S. C., Chou, H. C., Chiang, B. L. & Chen, C. M. CTGF upregulation correlates with MMP-9 level in airway remodeling in a murine model of asthma. *Arch. Med. Sci.* **13**, 670–676 (2017).
15. Ray, R. J., Furlonger, C., Williams, D. E. & Paige, C. J. Characterization of thymic stromal-derived lymphopoietin (TSLP) in murine B cell development in vitro. *Eur. J. Immunol.* **26**, 10–16 (1996).
16. Ito, T., Liu, Y. J. & Arima, K. Cellular and molecular mechanisms of TSLP function in human allergic disorders-TSLP programs the “Th2 code” in dendritic cells. *Allergol. Int* **61**, 35–43 (2012).
17. Hartgring, S. A. et al. Critical proinflammatory role of thymic stromal lymphopoietin and its receptor in experimental autoimmune arthritis. *Arthritis Rheum.* **63**, 1878–1887 (2011).
18. Nguyen, K. D., Vanichsarn, C. & Nadeau, K. C. TSLP directly impairs pulmonary Treg function: association with aberrant tolerogenic immunity in asthmatic airway. *Allergy Asthma Clin. Immunol.* **6**, 4 (2010).
19. Kaur, D. & Brightling, C. E. OX40/OX40 ligand interactions in T-cell regulation and asthma. *Chest* **141**, 494–499 (2012).
20. Kaur, D. et al. Mast cell-airway smooth muscle crosstalk: the role of thymic stromal lymphopoietin. *Chest* **142**, 76–85 (2012).
21. Ying, S. et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J. Immunol.* **174**, 8183–8190 (2005).
22. Lin, S. C., Cheng, F. Y., Liu, J. J. & Ye, Y. L. Expression and regulation of thymic stromal lymphopoietin and thymic stromal lymphopoietin receptor hetero-complex in the innate-adaptive immunity of pediatric asthma. *Int J. Mol. Sci.* **19**, 1231 (2018).
23. Zhou, B. et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat. Immunol.* **6**, 1047–1053 (2005).
24. He, R. & Geha, R. S. Thymic stromal lymphopoietin. *Ann. N. Y. Acad. Sci.* **1183**, 13–24 (2010).
25. Doras, C. et al. Lung responses in murine models of experimental asthma: value of house dust mite over ovalbumin sensitization. *Respir. Physiol. Neurobiol.* **247**, 43–51 (2018).
26. Gauvreau, G. M. et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N. Engl. J. Med.* **370**, 2102–2110 (2014).
27. Liu, Y. J. Thymic stromal lymphopoietin and OX40 ligand pathway in the initiation of dendritic cell-mediated allergic inflammation. *J. Allergy Clin. Immunol.* **120**, 238–244 (2007).
28. Halwani, R., Al-Muhsen, S. & Hami, Q. Airway remodeling in asthma. *Curr. Opin. Pharmacology* **10**, 236–245 (2010).
29. Broide, D. H. Immunologic and inflammatory mechanisms that drive asthma progression to remodeling. *J. Allergy Clin. Immunol.* **121**, 560–570 (2008).
30. Alcorn, J. F. et al. Transforming growth factor-beta1 suppresses airway hyperresponsiveness in allergic airway disease. *Am. J. Respir. Crit. Care Med.* **176**, 974–982 (2007).
31. McMillan, S. J., Xanthou, G. & Lloyd, C. M. Manipulation of allergen-induced airway remodeling by treatment with anti-TGF-beta antibody: effect on the Smad signaling pathway. *J. Immunol.* **174**, 5774–5780 (2005).
32. Makinde, T., Murphy, R. F. & Agrawal, D. K. The regulatory role of TGF-beta in airway remodeling in asthma. *Immunol. Cell Biol.* **85**, 348–356 (2007).
33. Serralheiro, P. et al. Variability of MMP/TIMP and TGF-beta1 receptors throughout the clinical progression of chronic venous disease. *Int J. Mol. Sc.* **19**, E6 (2017).
34. Ohbayashi, H. & Shimokata, K. Matrix metalloproteinase-9 and airway remodeling in asthma. *Curr. Drug Targets Inflamm. Allergy* **4**, 177–181 (2005).
35. Moore-Smith, L. D., Isayeva, T., Lee, J. H., Frost, A. & Ponnazhagan, S. Silencing of TGF-beta1 in tumor cells impacts MMP-9 in tumor microenvironment. *Sci. Rep.* **7**, 8678 (2017).
36. Kabata, H. et al. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. *Nat. Commun.* **4**, 2675 (2013).
37. Corren, J. et al. Tezepelumab in adults with uncontrolled asthma. *N. Engl. J. Med.* **377**, 936–946 (2017).
38. Zhang, W. X. & Li, C. C. Airway remodeling: a potential therapeutic target in asthma. *World J. Pediatr.* **7**, 124–128 (2011).