Transtracheal Instillation of Anti-TSLP Antibody Alleviates Airway Inflammation and Airway Hyperresponsiveness in OVA-Induced Asthma Model Mice

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Research Article

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Abstract

Background

Thymic stromal lymphopoietin (TSLP) is a mainly epithelial cell-derived cytokine that may be important in initiating T2 allergic inflammation. Anti-TSLP antibody inhibit allergic inflammation. Previous animal studies have evaluated the anti-allergic efficacy of injecting anti-TSLP antibody intraperitoneally, subcutaneously, or intravenously. However, transtracheal instillation, a less invasive route for anti-TSLP antibody administration, is hitherto unstudied. This study evaluates the efficacy of transtracheally instilling anti-TSLP antibody in inhibiting airway inflammation and hyperresponsiveness in OVA-induced asthma model Balb/c mice.

Methods

Balb/c mice were randomly divided into four groups: the control group, the OVA-induced asthma model group, the anti-TSLP mAb treatment group (TSLP mAb group), and the IgG2a mAb control group (IgG2a mAb group). Each group contained nine to eleven mice. Mice in the asthma model group were sensitized with OVA to trigger allergic responses and were treated with transtracheal instillation of normal saline. Mice in the TSLP mAb group received transtracheal instillation of anti-TSLP mAb, while mice in the IgG2a group received transtracheal instillation of IgG2a mAb. Both of these groups were then subjected to OVA challenge. Airway responsiveness was measured as an enhanced pause (Penh) using noninvasive plethysmography. The severity of inflammation was evaluated by histopathological examination using the Underwood assessment of the lung sections. Changes in expression of TSLP, TSLP receptor (TSLPR), T-box transcription factor (T-bet), GATA binding protein 3 (GATA3) and forkhead box protein P3 (Foxp3) were assessed using RT-PCR and immunohistochemical staining.

Results

Airway hyperresponsiveness and infiltration of airway inflammatory cells in the TSLP mAb group were significantly reduced, compared with the OVA and the IgG2a mAb groups. Meanwhile TSLP, GATA3 mRNA expression and GATA3 protein levels were significantly decreased in the TSLP mAb group, compared with the OVA and the IgG2a mAb groups (P 0.05). No significant differences were observed in either T-bet or Foxp3 in the lung section of mRNA and protein expression among these four groups (P 0.05).

Conclusion

Transtracheal instillation of anti-TSLP antibody attenuated lung inflammation and airway hyperresponsiveness in OVA-induced asthmatic mice, possibly through downregulation of perivascular and peribronchiolar lymphocytes, neutrophils and GATA3 expression in the airway.

Background

Allergic asthma is characterized by reversible airflow obstruction, airway hyper-responsiveness (AHR) and chronic airway inflammation. It is mainly driven by $CD4^+$ Th2 cells, which produce Th2-type immune responses[1]. The airway epithelium is a pivotal regulator of innate and Th2 immunity, which has a central role in asthma pathogenesis. GATA3 belongs to the GATA family of zinc-finger transcription factors and has two highly conserved C4-type zinc-fingers. It is crucial for the development and/or function of Th2 cells and group 2 innate lymphoid cells (ILC2s)[2].

Thymic stromal lymphopoietin (TSLP) is a mainly epithelial cell-derived cytokine of the IL-2 cytokine family. Studies show that TSLP production increases in response to protease allergens, viruses and bacteria [3]. These stimuli activate TSLP receptor (TSLPR)-expressing dendritic cells to induce Th2 cell polarization. Meanwhile *Tslpr*-deficient mice showed significant suppression of OVA-induced type 2 inflammation. Overexpression of TSLP in mice could cause type 2 airway inflammation associated with infiltration of Th2 cells in murine lungs and increased serum IgE levels[4].

Recent studies reported that TSLP plays important roles in orchestrating structural mechanisms relevant to asthma by altering the function of airway smooth muscle cells（ASMCs）[5], indicating that TSLP could promote airway inflammation through cross-talk between mast cells and airway structural cells[6]. Meanwhile, it has been proposed that TSLP released by lung epithelial cells might promote airway remodeling by employing activation of fibroblasts[7, 8].

Several investigations demonstrated that inhibition of TSLP could potentially alleviate airway inflammation. Both Toluene diisocyanate (TDI) exposed workers and TDI-induced asthma model mice showed increased levels of IgE and TSLP expression in lungs as well as allergic airway inflammation, the latter was alleviated by intraperitoneal administration of anti-TSLP antibody[9].

TSLP expression is elevated in asthmatic patients compared with healthy individuals in lung epithelium, airway smooth muscle and epithelial cells, serum and bronchoalveolar lavage fluid [10, 11]. Furthermore, the level of TSLP expression in patients with asthma is correlated with airway obstruction and disease severity[12]. Moreover, some recent clinical studies using anti-TSLP mAb have yielded promising results[13]. However, these studies mainly employ intraperitoneal or subcutaneous injections, at present, while inhaled medications remain the mainstream approach for clinical asthma treatment, since they are less invasive than injections. Transtracheal administration is an easier and more effective route for reversing antigen-induced asthma symptoms, bronchoalveolar lavage fluid (BALF) eosinophilia and IL-5 levels, compared to intraperitoneal or subcutaneous injection[14]. In this study, we tested the hypothesis that transtracheal instillation of anti-TSLP mAb is a new alternative approach for attenuating airway inflammation responses in mice with OVA-induced model asthma.

Methods

2.1. Mice sensitization and airway challenge

Forty-one SPF-grade female Balb/c mice (Guangzhou, China) were randomly divided into 4 groups including the control group, OVA group (OVA, chicken egg albumin, grade V, Sigma, St. Louis, USA), IgG2a mAb group, and TSLP mAb group. Each group contained nine to eleven mice. Mice sensitization and airway challenge methods are described in more detail in our previous report[14]. On days 0, 7, 14, the asthma model Balb/c mice were immunized with intraperitoneal injection of 100 μl of OVA/2mgAl(OH)3 20ug（Sigma, St. Louis, USA ）. On days 24–28, all groups were challenged intranasally with 20 μl of either 1% OVA or normal saline, for three days, followed by aerosol inhalation of 1% OVA solution for 30 minutes the next two days. Normal saline（NS）was used in the control group. The IgG2a mAb（R&D Systems China Co., Ltd）and the TSLP mAb （R&D Systems China Co., Ltd）groups were treated with IgG2a mAb or anti-TSLP mAb via transtracheal instillation before the OVA challenge.

2.2. Airway responsiveness to MCh

Airway responsiveness was measured 24h after the last aerosol exposure by recording respiratory pressure curves using barometric unrestrained whole-body plethysmography (WBP) (Buxco, Willmington, USA) in response to inhaled methacholine (MCh, Sigma, St. Louis, USA). In conscious and unrestrained mice, airway responsiveness was expressed as the enhanced pause (Penh). Mice were briefly placed in a whole-body chamber, to get average basal readings over a 3-min period. Subsequently, increasing doses of methacholine (0–100mg/ml) were aerosolized for 3-min, and the readings were obtained at the average for 3-min after each nebulization[15].

2.3. Histopathological and immunohistochemical staining assessments of murine lungs

Murine lungs were fixed in 4% paraformaldehyde (Sigma, St. Louis, USA) for 24h. Tissues were then paraffin-embedded (Sigma, St. Louis, USA) and cut into 5-μm sections. Sections were stained with hematoxylin and eosin (Sigma, St. Louis, USA) to assess inflammatory infiltration, edema, and epithelial damage. Pictures were then applied for the Underwood assessments. The Underwood score was assessed to evaluate airway inflammation[16] on a scale from 0 (normal) to 5 (severe). Each specimen was scored by two observers and a histopathologist. For immunohistochemical staining, lung tissues were incubated with primary antibody against GATA3、T-bet and Foxp3 (1:100; Abcam, Cambridge, United Kingdom) as previously reported[17].

2.4. Real-time polymerase chain reaction (PCR)

Lung tissues were gently ground with a plunger of a syringe and a stainless-steel filter mesh; intrapulmonary airways were then isolated carefully under a stereoscopic microscope using microsurgical instruments. Total RNA was isolated according to the protocol for TRIzol (Sigma, St. Louis, USA) and then reverse transcribed into cDNA using the Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit (Themo, USA). Real-time PCR was performed with the use of a QuantiFast SYBR Green PCR Kit (Invitrogen, Carlsbad, USA) on a Roche Light Cycler 480II system. Target gene expression was normalized to GADPH using the $2^{-\triangle\triangle}$ct method[18].

2.5. Statistical analysis

The data were expressed as the mean ± SEM. Statistical analyses (two-sample t-test, one-way ANOVA, and two-way ANOVA with Tukey`s post-tests) were performed with GraphPad Prism 8.2 (GraphPad, La Jolla, CA, USA). $P < 0.05$ was used to indicate a statistically significant difference.

Results

3.1. Changes of Penh values after transtracheal instillation of anti-TSLP antibody in OVA-induced asthma mice

To investigate the role of anti-TSLP mAb on airway function, airway hyperresponsiveness was measured by whole-body plethysmography with increasing doses of methacholine. As shown in Figure 1, Penh values in the OVA group were significantly increased compared with the control group (*P*0.05). Penh values in the anti-TSLP mAb group were significantly lower than those of the OVA group*P*0.05). There was no significant difference of Penh values between the IgG2a mAb group and the OVA group*P*0.05.

3.2. Histopathological examination of murine lung

The Hematoxylin and eosin staining revealed infiltration of inflammatory cells around the airway, bronchioles, and blood vessels; inflammatory cells were mainly lymphocytes and a few neutrophils, thickening of the bronchi, and alveolar and airway epithelium injury in OVA-induced mice, but not shown in the control group Figure2(a, b). These phenomena resolved substantially after anti-TSLP mAb treatment, but not after IgG2a mAb treatment Figure2(c, d). Presentation of inflammation degree in the lungs of the mice by H&E staining was done by the Underwood assessment, shown in figure 2(e) below.

3.3. The significance of mRNA changes after transtracheal instillation of anti-TSLP antibody in OVA-induced asthma mice

There was a marked, enhanced relative expression of lung TSLP and GATA3 mRNA in the OVA group compared with the control group, shown in figure 3 below. This enhancement was suppressed in the anti-TSLP mAb group ($P< 0.05$), but not in the IgG2a mAb group. Lung TSLPR mRNA expression in the control group was less than in the other three experimental groups ($P<0.05$), but there was no significant difference among these three groups ($P>0.05$). However, there were no statistically significant differences in lung T-bet and Foxp3 mRNA relative expression among these four groups ($P>0.05$).

3.4 Immunohistochemical staining of murine lung for GATA3, T-bet and Foxp3.

Immunohistochemical staining aera of murine lung tissues (Fig4) revealed that the intensity and distribution of GATA3 protein in the OVA-induced group were greater than those in the control group. Compared to the OVA group, the proportion of GATA3 positive cells was lower in the anti-TSLP mAb group, but not in the IgG mAb group. We also detected the differences in protein expression of T-bet and

Foxp3, which we assumed be related to the OVA-induced asthma pathological process, but staining showed that this was not the case. Each stain was repeated 3 times for each group.

Discussion

These data suggested transtracheal instillation of anti-TSLP antibody in the OVA-induced asthma model mice had inhibitive effects on airway inflammation and hyperresponsiveness. Measurement of respiratory parameters by whole-body plethysmography showed that OVA-induced asthma model mice treated with transtracheal instillation of anti-TSLP mAb had significantly decreased airway hyperresponsiveness, compared to untreated OVA-induced asthma model mice, which was in line with Chen`s study[19]. Airway smooth muscle contraction mediates airway narrowing. Several recent papers have suggested a role for TSLP in altering the function of airway smooth muscle cells and promoting airway remodeling by employing activation of fibroblasts. Therefore, the observed decrease of AHR after transtracheal instillation of anti-TSLP antibody may act through reducing airway TSLP concentration.

OVA-induced lung injury and IgE production mimic asthmatic inflammation. The degree of lung injury in this study was shown by H&E staining using the Underwood assessment. Our results suggested that administration of anti-TSLP mAb might ameliorate airway inflammation, lymphocytes and neutrophils infiltration in asthmatic mice. TSLP can differentiate naive $CD4^+$ T lymphocytes into Th2 cells and help them produce IL-4, IL-5, IL-13, while reducing the expression of Th1 related interferon-γ (IFN-γ)[20]. Based on these results, we successfully established an animal model of allergic asthma mice and measured the relevant immune response indicators including T-bet, GATA3, Foxp3 mRNA, and their protein changes to figure out potential mechanisms of action for anti-TSLP mAb transtracheal instillation.

T-bet, GATA3, Foxp3 are important transcription factors in Th1, Th2, and Treg cells, respectively. They indirectly reveal the direction of Th0 cell differentiation. Our data from RT-PCR showed that TSLP had a priming effect on mediated expansion and function of Th2 cells which was consistent with Rochman`s study [20, 21]. Under normal physiological conditions, GATA3 expression levels in prethymic progenitor cells are very low and can be upregulated by Notch signaling on migration of the cells into the thymus to be matured[2]. Anti-TSLP mAb might directly or indirectly participate in altering this process. However, our results showed no significant difference on Foxp3 mRNA expression among the four groups, which is in conflict with Nguyen`s study [22]. They suggested that TSLP was able to reduce the anti-inflammatory function of Tregs cells and hence potentiated T2 (eosinophilic allergic) inflammation in asthma. Our experiments are preliminary, and the mechanisms of action of observed effects are still unclear. Further studies are needed to identify additional signaling pathways that might take part in Th1 and Treg regulation.

Since proteins indicate physiological functions more directly than mRNA, we did further immunohistochemical staining of the murine lung. The results revealed that anti-TSLP mAb suppressed the up-regulation of GATA3 protein. It supported that Th2 cytokines are involved in OVA-induced allergic responses. However, immunohistochemical staining is a semi-quantitative measurement to detect protein

change. Further studies on inflammation biomarkers or cytokines from BALF and blood analysis by Western blot and ELISA could provide more quantitative data on protein level.

We provide in-vivo evidence that transtracheal instillation of anti-TSLP mAb inhibited OVA-induced airway inflammation and downregulated GATA3 mRNA and protein expression. The results suggested that transtracheal instillation of anti-TSLP mAb might be a promising new method to treat allergic asthma. However, it is unclear whether the inflammatory inhibition was due to the direct effect of the anti-TSLP mAb. Further study is needed to clarify the mechanisms and related phenomena.

Conclusion

Transtracheal instillation of the anti-TSLP mAb significantly alleviated the airway hyperresponsiveness and the inflammation severity of the lung in an asthmatic mice model induced by OVA. Anti-TSLP mAb may downregulate lymphocytes, neutrophils and GATA3 expression in the airway.

Abbreviations

TSLP, Thymic Stromal Lymphopoietin; TSLPR, Thymic Stromal Lymphopoietin receptor; GATA3，GATA binding protein 3，T-bet , T-box transcription factor; Foxp3, forkhead box protein P3; T2 inflammation, Type 2 inflammation，OVA，ovalbumin，AHR，airway hyperresponsiveness.

Declarations

Ethics approval and consent to participate

All mouse procedures described were approved by the Research Ethics Committee Guangdong Provincial People`s Hospital, Guangdong Academy of Medical Sciences (No. GDREC2019219A), and were performed in accordance with *Guide for the Care and Use of laboratory Animals.* The study was carried out in compliance with the ARRIVE guidelines.

Consent to Publish

Not applicable

Availability of data and materials

The data sets used and\or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

XFF, XWC, JXM, and JW planned this study. XFF and JQ performed animal experiments. DFL and SHL guide the experiments. XWC, XFF, JLL, JW contributed to the preparation of the manuscript. XLL prepared the figures. All authors have read and approved the final manuscript. XWC and XFF contributed equally to this work. JXM and JW are the corresponding authors.

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Figures

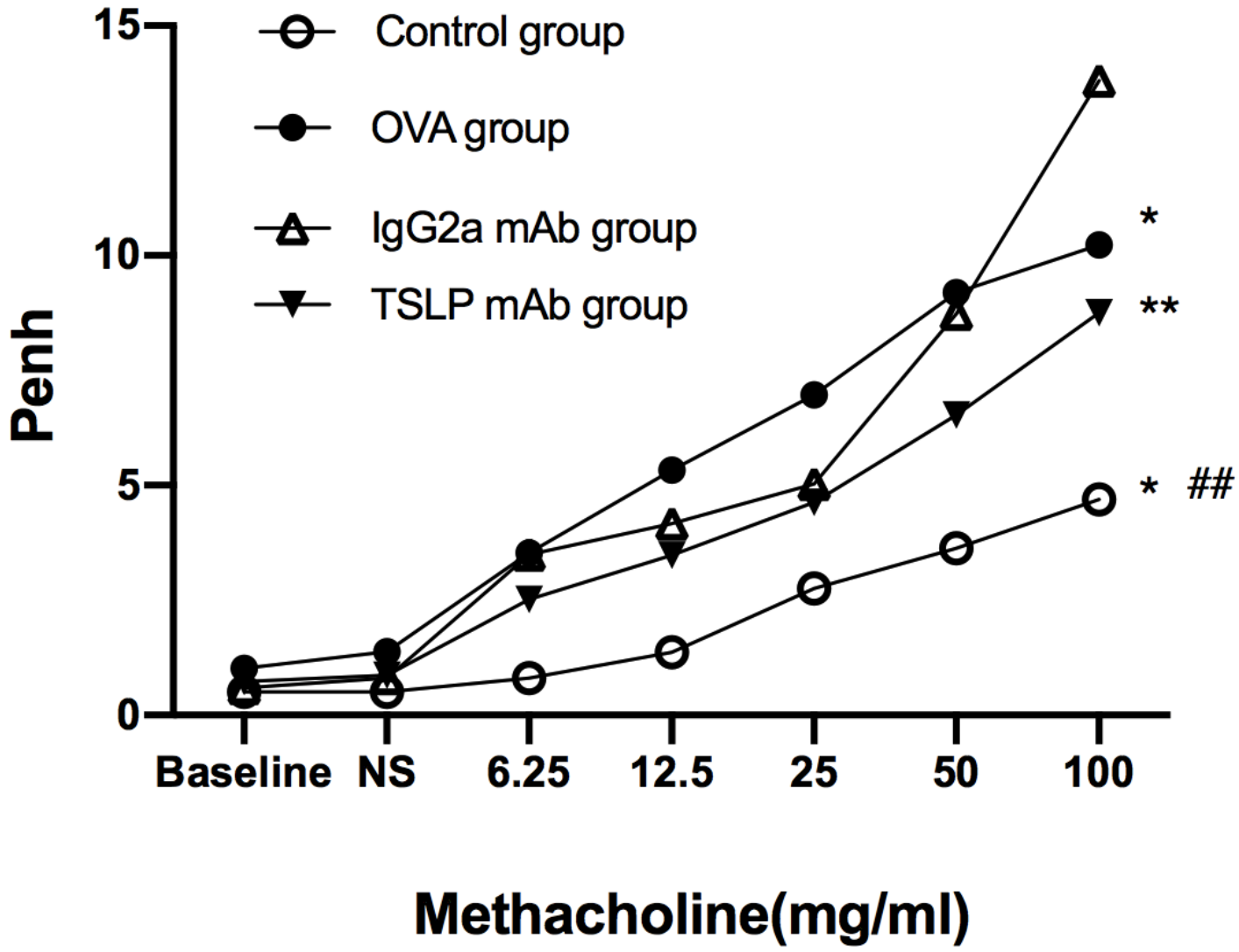
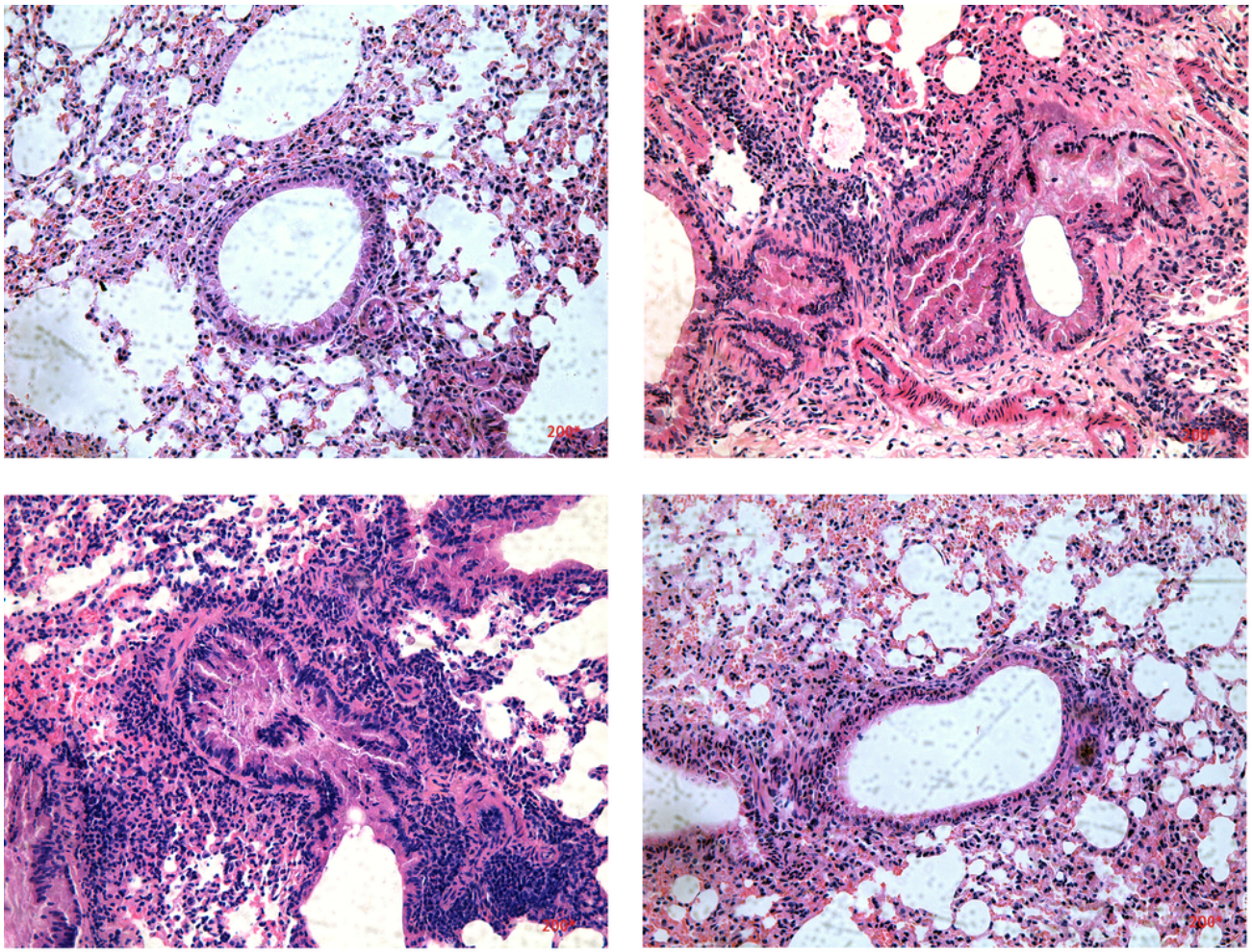


Figure 1

Results from whole-body plethysmography in awake, freely moving mice are shown. Exposure to OVA increases airway hyperresponsiveness (AHR) *P < 0.05 and ** P < 0.01. “*” indicate comparison with the control group and “#” indicate comparison with the OVA group.



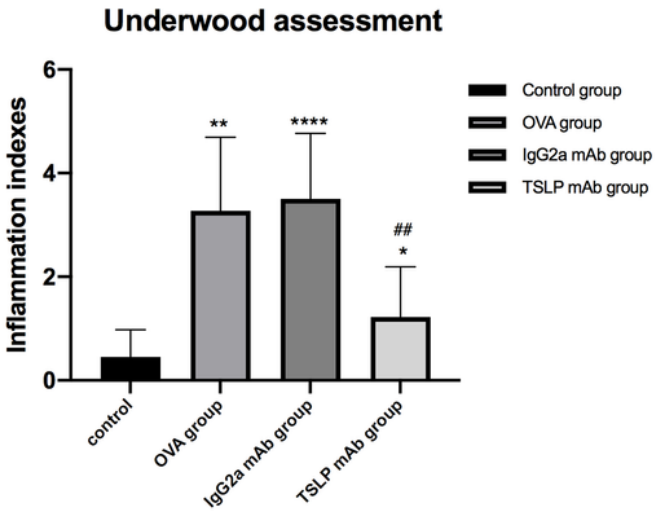


Figure 2

The histopathological changes in the lung tissue examined by H&E staining are shown. Lung tissue was taken 24 hours after the last OVA challenge. In (a), there is normal bronchiole structure. In (b) and (c), there is edema fluid in the alveoli cavities and bronchiole and extensive inflammatory cell infiltration around the small blood vessels and bronchiole. In (d), inflammatory conditions were ameliorated using transtracheal instillation of anti-TSLP mAb. (e) shows the Underwood assessment for all the four groups,

according to the degree of perivascular and peribronchiolar eosinophilia, edema, and epithelial damage. Values are expressed as means ± SEM. * P < 0.05 and ** P < 0.01, *** P□0.001, n = 9. “*” indicate comparison with the control group and “#” indicate comparison with the OVA group.

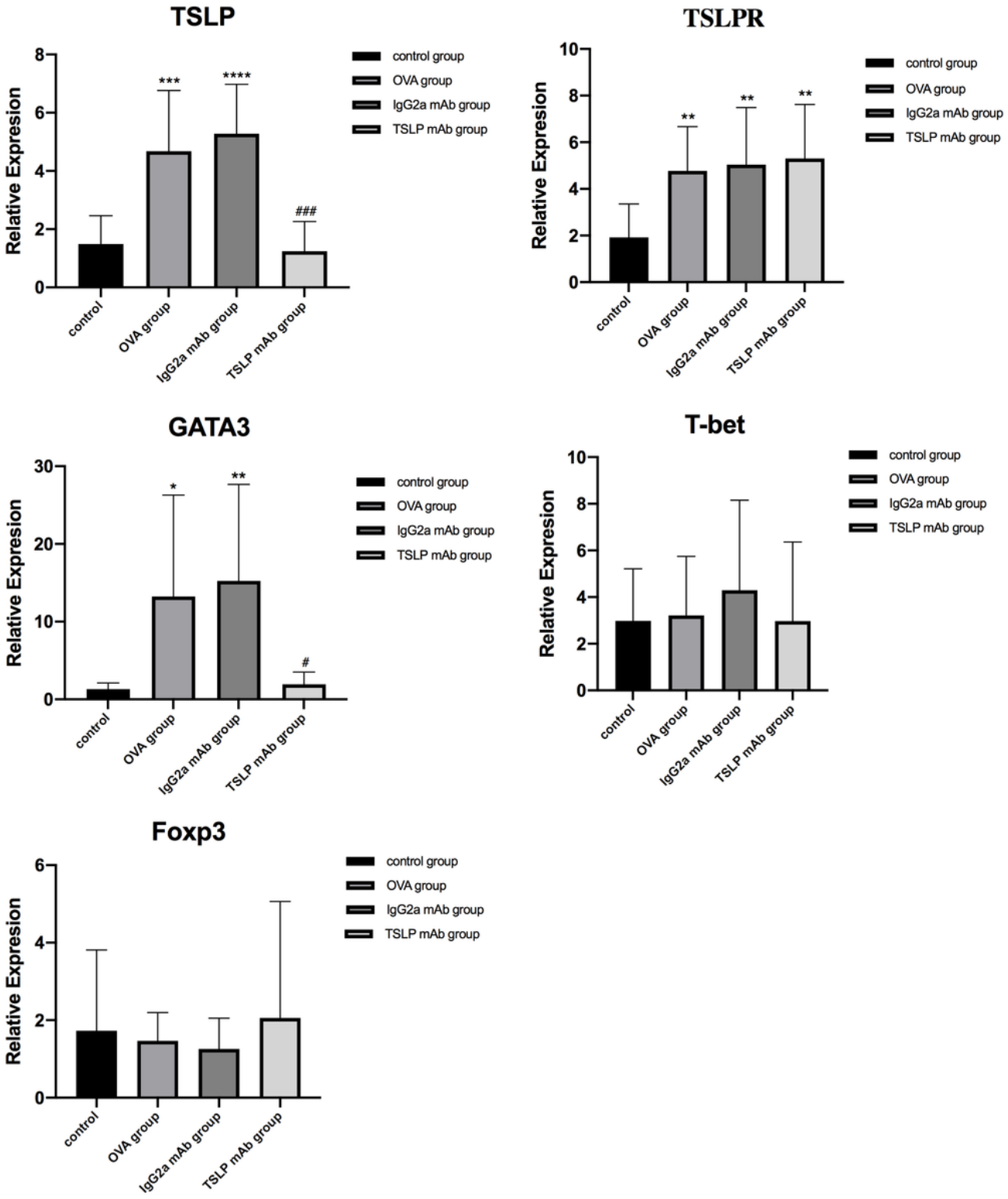


Figure 3

TSLP, TSLPR, GATA3, T-bet, Foxp3 mRNA expression in lung tissue. Values are expressed as the means ± SEM. * P < 0.05 and ** P < 0.01, n = 9. “*” indicate comparison with the control group and “#” indicate

comparison with the OVA group.

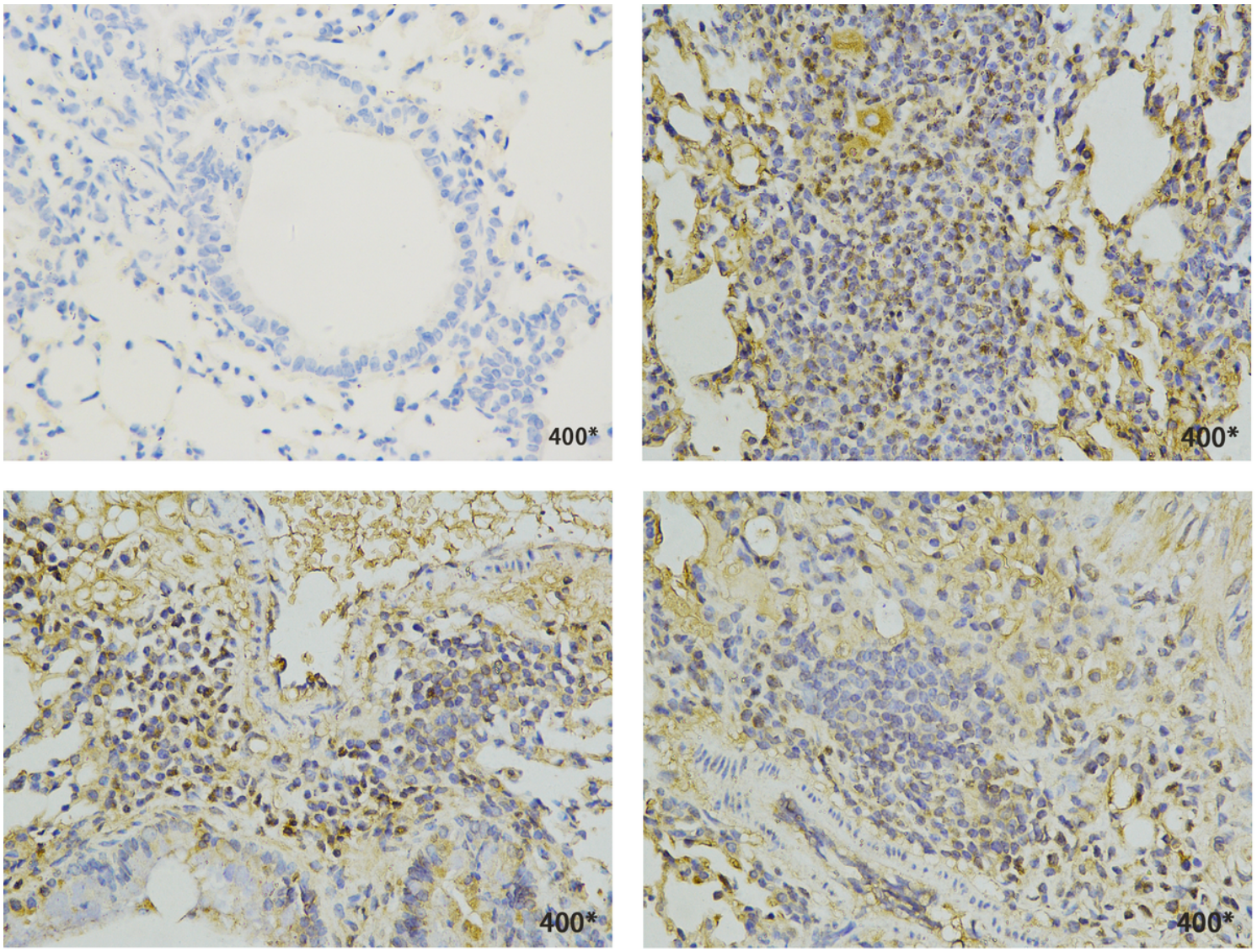


Figure 4

Immunohistochemical staining of the murine lung for detecting GATA3 protein (a,b,c,d). GATA3-positive cells, indicating GATA3 expression, were stained brown.