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Local blockade of TSLP receptor alleviated allergic disease by regulating airway dendritic cells

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KEYWORDS

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Abstract Thymic stromal lymphopoietin (TSLP) emerges as a central mediator of T helper cell (Th)2-dominant allergic diseases. However, the role of TSLP receptor (TSLPR) in allergen-induced Th2 priming, and the effects of TSLP signaling blocking on the development of asthma remain unclear. Here we showed that allergen challenge caused a rapid accumulation of TSLP in the airways of asthmatic mice, correlating well with eosinophils counts and interleukin (IL)-5 productions. When TSLP signaling was blocked by intratracheal administration of anti-TSLPR antibody before sensitization, eosinophilic airway inflammation, goblet cell hyperplasia and Th2 cytokines productions were significantly reduced. The alleviating effects of TSLPR blocking were achieved by inhibition of maturation and migration of airway dendritic cells (DCs), as well as their abilities of initiating CD4+T cell responses. Thus, local application of anti-TSLPR prevented Th2-mediated airway inflammation, at least partly, by regulating DCs function, which might be exploited to develop novel treatments for asthma.

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Introduction

The participation of genetic, environmental factors and immune regulation complicate the pathogenesis of allergic asthma, a chronic respiratory disease characterized by airway inflammation and airway hyperresponsiveness (AHR). However, a dysregulated Th2 polarization and expansion has been presumed to play critical roles in the development of the disease, and mechanisms underlying the

Th2 differentiation in allergen-induced airway responses are presently under intensive investigation [1,2].

Strategically positioned at high antigen-exposed sites, airway DCs have been regarded as critical initiators of allergic inflammation, not only by their abilities of processing and presenting inhaled antigenic information to the draining pulmonary lymph nodes (LNs), but also by their capabilities of activating naïve T cells, and even determining the type of immune response in the afflicted areas [3,4]. It is recognized that the abilities of DCs to direct different Th responses largely depend on the signals DCs receive from both the invading antigens as well as the tissue

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microenvironments factors [5,6]. Compelling data about toll-like receptors (TLRs) on DCs have illuminated the mechanisms by which microbe-molecules drive DC-mediated Th1 differentiation. However, which factors and how these factors condition DCs to induce Th2 differentiation are relatively less known to date. Delineating the signaling through which DCs prime T cells to differentiate into Th2 effector cells is central to understand the pathogenesis of allergic asthma and develop efficient treatment for the disease.

Thymic stromal lymphopoietin (TSLP) emerges as one of the most powerful mediators to induce DC-mediated Th2 response [7,8]. It endows DCs with the ability to stimulate naïve T cells and promote Th2 differentiation by triggering the production of proallergic cytokines (IL-4, IL-5, IL-13). Also, TSLP activates CD11c⁺ DCs to produce chemokines CCL17 and CCL22, recruiting Th2 cells to the draining LNs. Specific expression of TSLP in atopic dermatitis lesions and asthmatic airway, and the induction of pronounced airway inflammation in the *Tslp* transgene mice implicated this novel cytokine in allergic diseases [9–11]. Further evidence to support the importance of TSLP signaling in allergic airway inflammation was derived from TSLP receptor (TSLPR)-deficient (*Tslpr*^{−/−}) mice which exhibited remarkably decreased airway inflammation and goblet cell hyperplasia upon ovalbumin (OVA) challenge in comparison to wide type [12].

TSLPR is a novel type 1 cytokine receptor, which, combined with IL-7 receptor- α (IL-7R α), constitutes TSLP receptor complex [13]. TSLPR possesses a tyrosine residue near its carboxyl terminus, which can associate with phosphorylated Stat5 and mediate multiple biological functions when engaged with TSLP [14]. The above studies illuminated the significance of TSLP/TSLPR in allergic diseases, and suggest a new therapeutic target for the treatment of allergic diseases, but the data from *tslp* overexpression or *Tslpr*^{−/−} mice couldn't exclude the systematic effects which have been evidenced by previous studies to be associated with some immunological disorders [15–17]. Moreover, previous studies demonstrated that intrapulmonary antagonizing of functional molecules on effector cells was effective in abrogating the cardinal features of asthma, while intraperitoneal regulating of circulating leukocytes only partially inhibited airway responses [18–20]. Here we adopted local administration of anti-TSLPR antibody in allergen-primed asthmatic mice to assess the effects of TSLPR blocking in the development of airway inflammation, and sought to identify the involved mechanism, with more attentions placed on DCs, which is believed to be critical in Th2-dominated allergic disease.

Materials and methods

Mice

Female 8–10 wk old BALB/c were purchased from Zhejiang Laboratory Animal Center. The DO11.10 TCR (specific for OVA 323–339peptide) transgenic mice were obtained from Jackson Laboratory. All the mice were bred and maintained in a specific pathogen-free facility at the Animal Resources Center, Hangzhou Normal University (No. SYXK Zhe 2005-

0070). All the experiments conform to the principles for the care and use of animals in biomedical research.

OVA sensitization and airway challenge

Three groups of mice ($n=8$ in each group) were sensitized on days 1 and 14 by intraperitoneal injection of 100 μ g OVA (grade V; Sigma) emulsified in 1.25 mg aluminum hydroxide in a total volume of 200 μ l PBS. For nonsensitized control mice, PBS plus aluminum hydroxide without OVA was injected. Anesthetized mice were challenged intranasally (i.n.) with 100 μ g OVA in 40 μ l PBS for 3 days starting from day 21 [9]. Mice were analyzed 24 h after the last challenge. In the TSLPR blocking experiment, thirty minutes before each OVA sensitization, mice were anesthetized using avertin and received an intratracheal (i.t.) injection of vehicle, or 20 μ g of anti-TSLPR (AF546, R&D System) or control IgG (AB-108-C, R&D System) [18,20]. The dose of blocking antibody was predetermined by staining analysis of lungs from mice receiving 10–40 μ g antibody respectively [18].

BAL and histological analysis

Twenty-four hours after the last OVA exposure, BAL was performed 3 times with 0.75 ml Ca²⁺ and Mg²⁺ free HBSS supplemented with 0.1 mM sodium EDTA. BAL fluid samples were centrifuged at 1400 rpm for 5 min to generate a cell pellet and a cell-free supernatant, which was frozen and stored at −80 °C until analyzed by ELISA. TSLP concentrations in BAL were quantified by TSLP-specific ELISA as described in 9. After red blood cells (RBCs) were lysed using ammoniumchloride lysis buffer, all the remaining cells were prepared by cytopspining (Cytospin 2, Shandon, Cheshire, U.K.) and staining with Diff-Quik (Baxter Healthcare, Miami, FL). Differential cell counts were performed on 300–400 cells in 6 different fields based on standard morphologic criteria at 40 $\times$ magnification. Absolute cell number was calculated by multiplying the total leukocyte number by the percentage of each population of interest.

For lung histology, nonlavaged lobe of lungs was resected, fixed, and embedded, and 5- μ m sections were prepared and stained with hematoxylin and eosin (H&E) for analysis of cellular infiltrates. Airway mucus was also analyzed by sequential staining with periodic acid and Schiff's reagent (PAS), and positive material was identified as magenta.

Evaluation of maturation and migration of pulmonary DCs

BALB/c mice were i.t. instilled 20 μ g of anti-TSLPR or Ig followed by an i.t. injection of 80 μ l OVA (10 mg/ml). Control mice received 80 μ l PBS. Lungs were collected and digested with collagenase III and DNase I. Suspended cells (95% viability) were stained with APC-CD11c, FITC-MHC class II, and PE-CD40, CD80 or CD86 mAbs (BD Pharmingen) and were then analyzed with a FACSCalibur flow [21,22].

To address migration of lung DCs, 80 μ l of FITC-OVA (10 mg/ml) with 20 μ g of anti-TSLPR or Ig, or control vehicle was administered i.t. into the mice. 40 h later,

peripheral (axillary and inguinal) and mediastinal LNs were collected, homogenized, and stained with PE-MHC class II and APC-CD11c Abs, and analyzed by flow cytometry for FITC positivity [22].

Naive T cell activation and differentiation, and splenocytes activation in vitro

Bone marrow cells were isolated and cultured in DCs medium as described [23]. 8-day DCs were collected and pulsed with OVA (10 μ g/ml) overnight, treated with vehicle or with 100 ng/ml TSLP (555-TS/CF, R&D), and in some experiments, anti-TSLPR or control Ig were also added in the last 2 h. Next day, DCs were collected, washed, and cultured with naive TCR transgenic CD4⁺T cells obtained from DO11.10 mice for 3 days at various ratios. Cell proliferation of naive T cells was assessed by ³H-thymidine (1 μ Ci/well) uptake in a 16-h pulse. To assess Th cell differentiation, supernatants from DC-T coculture were harvested and analyzed by ELISA (Biosource). For total splenocytes activation, splenocytes from DO11.10 mice were cultured with 5 μ g/ml of OVA_{323–339} peptide. Anti-TSLPR or control Ig was applied as indicated and 3 days later levels of IFN- γ in the culture were analyzed by ELISA.

Th2 priming induced by i.t. injection of bone marrow-derived mDCs

8-day DCs were cultured overnight in the presence of OVA, also with anti-TSLPR or control Ig added in the last 2–3 h. Non-adherent DCs were collected, washed and resuspended in PBS. For in vivo experiments, BALB/c mice were anesthetized and i.t. instilled with 2×10^6 DCs, and on days 10–12 mice were exposed to OVA aerosol for 30 min once per day and killed 24 h after the last dose [21,24].

Statistics

For all experiments, differences were evaluated for significance ($P < 0.05$) by the Student's 2-tailed t test for independent events, and the coefficient of correlations was calculated by statistical analysis using Microsoft Excel. Data are given as mean values $\pm$ SEM.

Results

Levels of TSLP were significantly increased in bronchoalveolar lavage fluid (BALF) of asthmatic mice

Using a mouse model of airway inflammation induced by OVA sensitization followed by three sequential daily OVA challenges, we investigated TSLP levels during allergic Th2 responses and the functional relevance of this cytokine for the disease. As shown in Fig. 1A, there was a significant elevation of TSLP levels in BALF from mice sensitized with OVA as compared with PBS-primed controls. Moreover, a rise in TSLP in OVA-sensitized mice, but not in PBS-sensitized mice, showed to correlate well with an increase in BALF eosinophils and IL-5 levels respectively (Figs. 1B, C), suggesting the strong association of TSLP with the pathogenesis of experimental asthma.

Local treatment with anti-TSLPR antibodies reduces airway inflammation, Th2 skewing and mucus production

As increased expression of TSLP in asthmatic mice was closely related with IL-5 production and eosinophils infiltration, we then asked whether blockade of TSLP signaling would decrease Th2 cytokines production and eosinophilic inflammation in asthmatic mice. To address this question, and also to evaluate the effects of TSLPR blocking in allergen-primed airway inflammation, we locally administrated a neutralizing antibody to TSLPR (or control Ig) before OVA sensitization [18,20]. As expected, there was an elevation of BAL total cells in OVA-sensitized mice upon subsequent OVA challenge, as compared with PBS-sensitized mice. However, pretreatment with TSLPR antibody led to a significant decrease in the number of BAL total cells as well as eosinophils and lymphocytes, in comparison with Ig-treated mice (Fig. 2A).

Along with this, local blockade of TSLPR resulted in a remarkable reduction in IL-4 and IL-5 levels while enhancing expression of Th1-associated cytokine interferon (IFN)- γ , as compared with mice receiving control Ig. Notably, TSLPR blocking also induced a slight up-regulation of IL-10, a cytokine presumed to have suppressive property in the immune response (Fig. 2B).

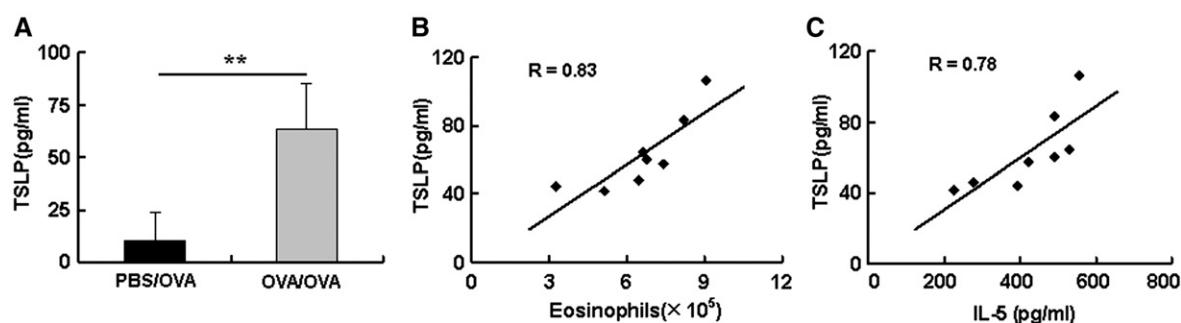


Figure 1 Levels of TSLP increase during asthmatic airway inflammation in mice. (A) TSLP levels, as analyzed by ELISA, in BALF of OVA- or PBS-sensitized mice. (B) A positive correlation between the levels of TSLP and eosinophils ($r = 0.8347$), and (C) a relatively lower correlation between TSLP and IL-5 levels ($r = 0.7845$) in BALF from OVA-sensitized mice. Data are mean $\pm$ SEM; $n = 8$ mice in each group. ** $P < 0.01$.

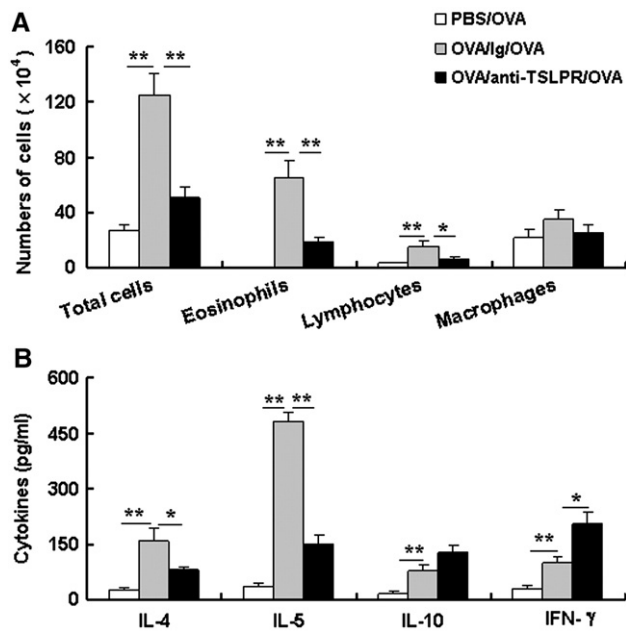


Figure 2 Neutralizing TSLPR suppresses allergic responses. OVA-sensitized and challenged mice received an i.t. injection of anti-TSLPR or control Ig treatment 30 min before each OVA sensation. (A) Total and differential cells counts and (B) Cytokine profile in BALF were analyzed 24 h after final OVA exposure. Data represent mean values $\pm$ SEM from 5 mice per group. * $P < 0.05$; ** $P < 0.01$.

To further characterize the effects of TSLPR blocking on OVA-induced airway inflammation, we also compared lung histology in different groups of mice. As shown in Fig. 3, OVA-primed mice developed dense perivascular and peribronch-

olar infiltrates of eosinophils and lymphocytes, together with abundant mucin production in epithelial cells, which were rarely detected in sham-sensitized mice. On the contrary, anti-TSLPR administration significantly alleviated airway inflammation, as we just observed little peribroncholar infiltrates, minor goblet cell metaplasia and presence of mucus in the airways. Collectively, local blockade of TSLPR in asthmatic mice substantially lessened the severity of allergic airway responses and tended to deviate from allergen-induced Th2 development.

TSLPR blockade at sensitization affects airway DCs maturation and migration

As DCs play a vital role in sensitization to inhaled antigen and then the development of asthma [3,4,6], whereas TSLP has been recognized as a potent activator of DCs [7,8], we therefore wondered if pulmonary DCs might be involved in anti-inflammatory effects of TSLPR inhibition in allergen priming. We first assessed the maturation of lung DCs, a prerequisite for initiating the immune response to inhaled allergens in the airway. As shown in Fig. 4A, pretreatment with anti-TSLPR induced a remarkable reduction in CD40, CD80, CD86, or MHC II expressions by pulmonary DCs in terms of mean fluorescence intensity (MFI), compared with those of Ig-treated mice (as DCs from PBS only mice were not obviously activated, the data were not shown), suggesting that maturation and activation of lung DCs during initial antigenic encounter were substantially affected by the inhibition of TSLPR signaling. Additionally, given that migration of DCs to the draining LNs is essential step for generating effector Th2 cells [3,22], we further assessed the migratory ability of DCs in response to allergen by enumerating the number of MHCII⁺ CD11c⁺ DCs carrying fluorescent

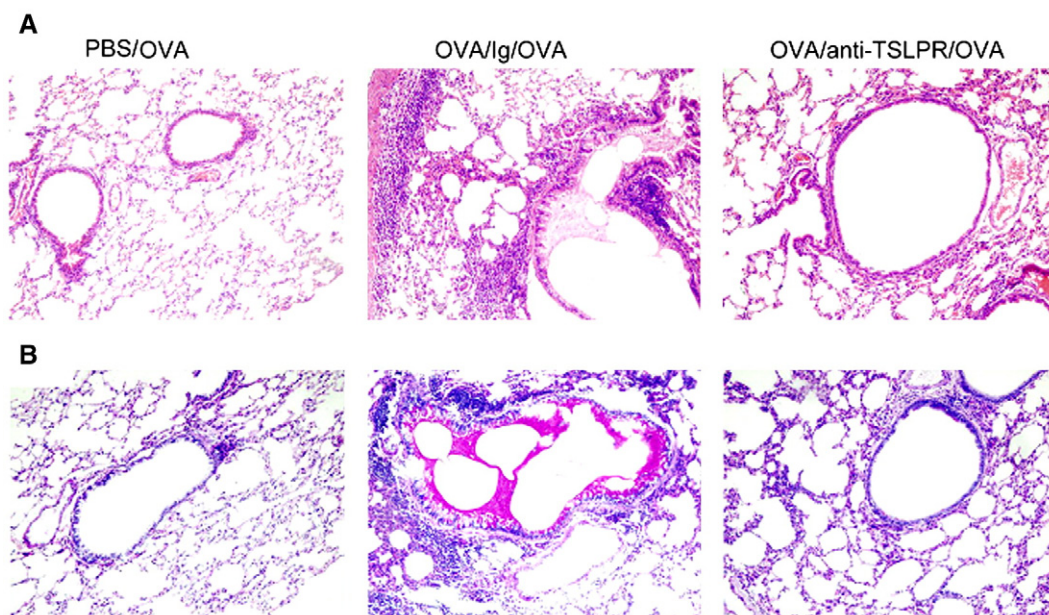


Figure 3 TSLPR inhibition alleviates lung inflammation. OVA-sensitized and challenged mice received an i.t. injection of anti-TSLPR or control Ig treatment 30 min before each OVA sensation, and histological examination of lung was performed 24 h after final OVA exposure. Lung sections were stained for cellular infiltrates with H & E (A) and mucus production with PAS (B). Original magnification, $\times 20$.

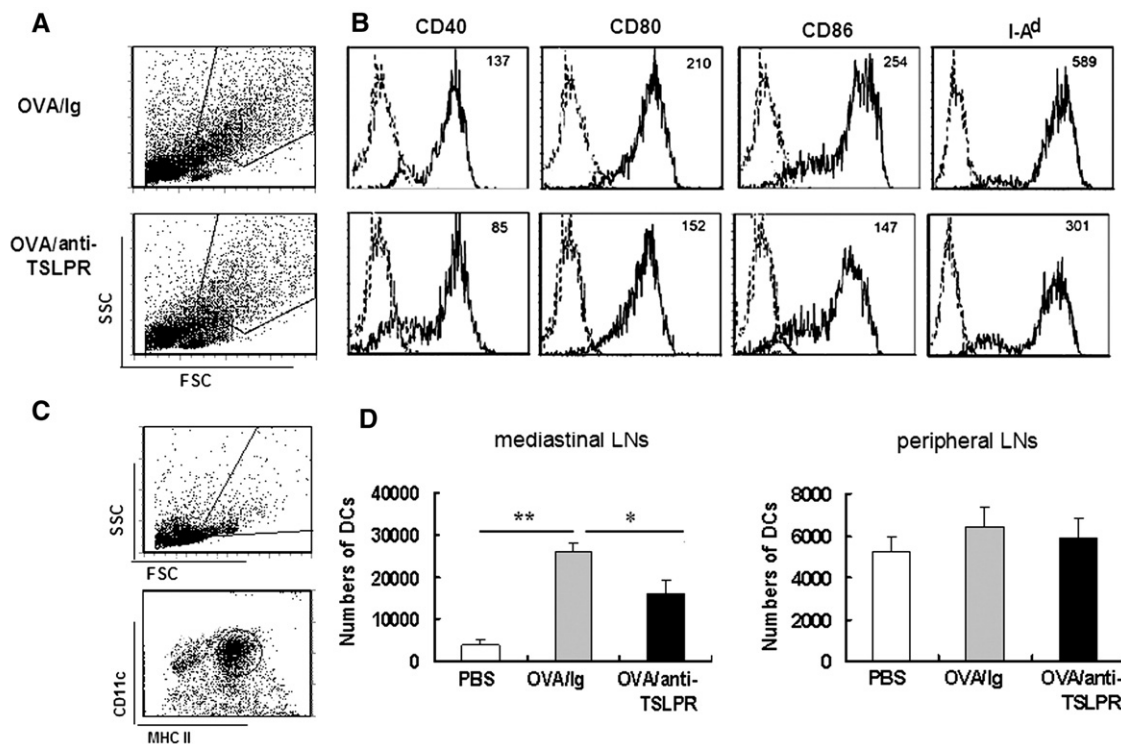


Figure 4 TSLPR blocking alters the maturation and migration of airway DCs. (A) Forward scatter (FSC)/side scatter (SSC) plots of lung cell suspensions from OVA/Ig or OVA/anti-TSLPR-treated mice. (B) Expression of CD40, CD80, CD86, and MHC II of lung DCs (CD11c⁺ MHC II⁺) were analyzed 24 h after OVA instillation. Numbers in plots represent mean fluorescence intensity (MFI) of lung DCs from Ig- or anti-TSLPR-treated mice (solid lines); dot lines mean isotype control. (C) Flow cytometric staining pattern of LN single cell suspensions of FSC/SSC plot, and DCs were identified as CD11c^{high} MHCII^{high} cells (D) Numbers of DCs migrating into peripheral or mediastinal LNs of PBS or FITC-OVA/Ig or FITC-OVA/anti-TSLPR-treated mice were assessed by flow cytometry. CD11c⁺ MHCII⁺ cells were also detected for the presence of FITC-OVA. Absolute number of cells are indicated as mean $\pm$ SEM. * P < 0.05; ** P < 0.01.

FITC-OVA cargo in LNs 40 h after instillation. Strikingly, there occurred a decrease in DC counts in mediastinal LNs (MLNs) of anti-TSLPR-treated mice compared with those of Ig-treated mice, while no significant differences in DC numbers in the peripheral LNs were observed between these two groups of mice (Fig. 4B), indicating that inhibition of TSLPR also exerted influences on migration of pulmonary DCs.

TSLPR neutralization affects the potential of TSLP-conditioned DCs to activate and polarize Ag-specific T cells

The above data established the preventing roles of TSPR inhibition in the airway response by inhibiting DCs' maturation and migration in response to allergen, but the effects of TSLPR blocking on Th2-polarization capability of DCs hadn't been investigated yet. Given the fact that respiratory tract DCs, in steady state or in inflammatory environment, are continuously replenished by a bone marrow (BM) output [25,26], we therefore utilized the coculture of BM-DCs and DO11.10 Th to examine the impacts of TSLPR blocking on DC function. Expectedly, TSLP treatment significantly enhanced the capability of OVA-DCs to activate naïve T cells, as shown by a rapid proliferation of OVA-specific T cells, which nevertheless was reversed with the addition of anti-TSLPR (Fig. 5A). Moreover, analysis of cytokines production in the coculture demonstrated that TSLP-conditioned DCs stimu-

lated T cells to produce large amount of IL-4, associated with decreased IFN- γ productions, as compared with control DCs. And this effect was also abrogated by pretreatment of anti-TSLPR on DCs (Figs. 5B, C), indicating the central role of TSLPR-mediated signaling in regulating DCs function.

To further characterize the physiological function of TSLPR, we also assessed the effect of TSLPR inhibition on the culture of total splenocytes, supposed to have constitutive and induced expression of TSLPR [27]. As shown in Fig. 5D, splenocytes from DO11.10 mice cultured in the presence of OVA produced moderate amount of IFN- γ , which was significantly up-regulated by inhibition of TSLPR. This phenomenon was in accordance with the previous observation from *tslpr*^{-/-} mice [12], suggesting the involvement of IFN- γ in the in vivo effects of TSLPR blocking.

Blocking TSLPR impairs the capability of mDCs to induce Th2 development in vivo

To get better understanding of the influence of TSLPR inhibition on the capability of DCs to prime Th2 differentiation in vivo, and to eliminate any direct effects of anti-TSLPR on effector cells in airway inflammation, we performed experiments in which OVA-pulsed mDCs were subject to anti-TSLPR treatment before they were adoptive transferred to mice airways. As expected, mice immunized with OVA-pulsed DCs showed a strong cellular recruitment of lymphocytes and

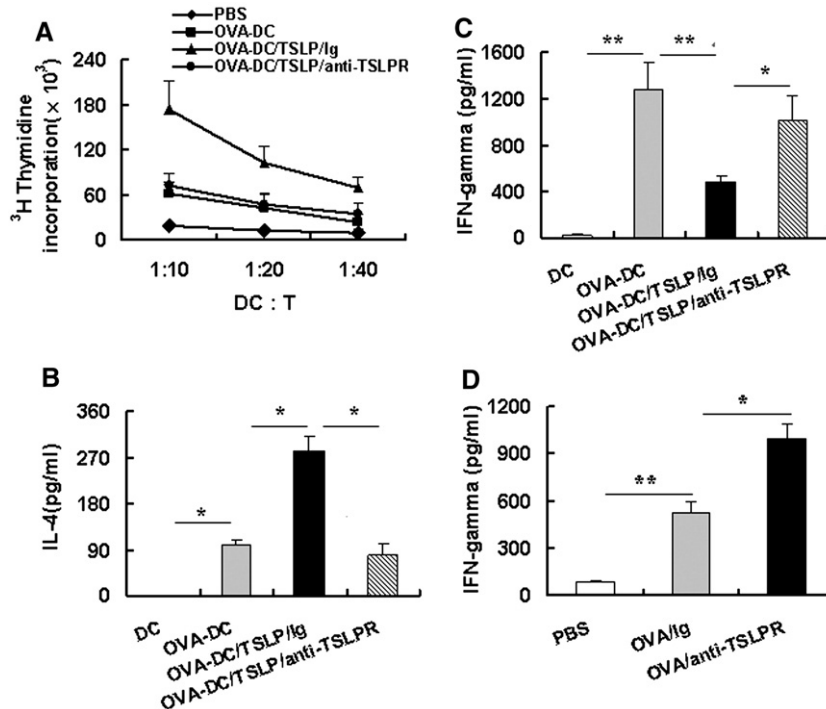


Figure 5 TSLPR blocking impairs the ability of DCs to prime Th2 responses in vitro. Bone marrow-derived DCs were incubated overnight in the presence of OVA and TSLP (100 ng/ml) or vehicle, and in some experiments, anti-TSLPR or control Ig were also added. Cells were then collected and cocultured with DO11.10 Th cells for 3 days. (A) [^3H] thymidine incorporation was determined. (B, C) Cytokine profiles in cell culture (DC:T=1:10) supernatants were assessed by ELISA. (D) Levels of IFN- γ in the culture of splenocytes from DO11.10 mice with or without anti-TSLPR were also analyzed. Data are shown as mean $\pm$ SEM. * P <0.05; ** P <0.01.

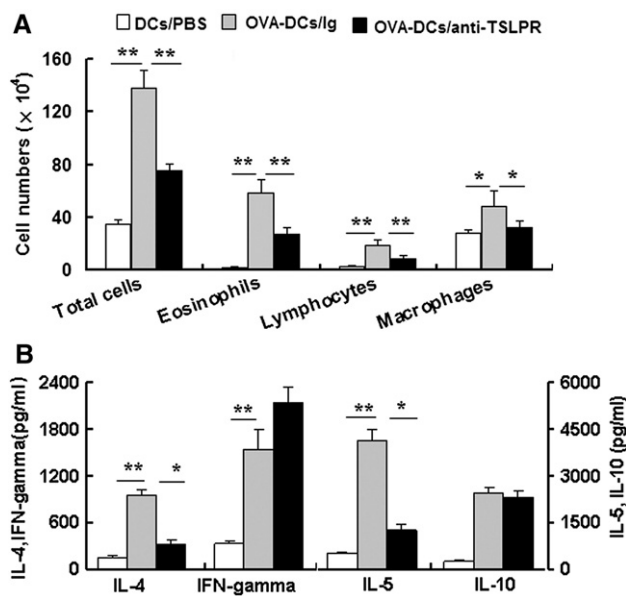


Figure 6 Blocking TSLPR on DCs reduces their capacities to prime Th2 responses in vivo. Mice were given an i.t. injection of Ig- or anti-TSLPR-treated, OVA-pulsed DCs, or unpulsed DCs on day 0 and then exposed to OVA aerosols on days 10–13. (A) BALF cell differential counts were assessed 24 h after final OVA exposure. (B) Cytokine levels in the supernatants of MLN cells restimulated in vitro for 4 days with OVA were analyzed by ELISA. Data are shown as mean $\pm$ SEM. * P <0.05; ** P <0.01.

eosinophils, compared with mice receiving unpulsed DCs. However, treatment of OVA-pulsed mDCs with anti-TSLPR significantly reduced the potential of these cells to induce eosinophilic airway inflammation and Th2 differentiation, as shown by decreased IL-4, IL-5 expressions along with a moderate elevation of IFN- γ (Figs. 6A, B). The data strongly confirmed that inhibition of TSLPR on DCs could restrain the potential of DCs to induce Th2 priming in asthmatic mice.

Discussion

Asthma is a multiple-step immune disorder characterized by the mass infiltration of various inflammatory cells including eosinophils, macrophages, mast cells, neutrophils and Th2 cells. Pulmonary DCs, however, play even more important roles as upstream instigators in the allergic inflammatory cascade, not only through their powerful abilities of processing and presenting inhaled antigen, but also through their unique properties of orchestrating immune responses [5,6], therefore they become the key to understand the pathogenesis of, and also to devise efficient treatment for allergic diseases. A growing body of experiments indicate that inhaled external agents like diesel exhaust particles, pollen-derived isoprostanes, induce allergic inflammatory responses by facilitating the activation and antigen transportation of airway DCs, as well as upregulating DCs' production of Th2-attracting chemokines and proinflammatory cytokines to establish an inappropriately Th2-oriented immune climate in the airway [28,29]. Also, tissue-derived

factors such as histamine, osteopontin and extracellular ATP contribute to the specific airway microenvironment directing DCs toward a proallergic or antiallergic function [30–32]. Among these TSLP is supposed to be a potent factor in driving DC-initiated Th2 response and closely linked with allergic diseases [8–12].

Here we confirmed that production of TSLP was significantly increased in asthmatic mice, and this increase was correlated well with elevated IL-5 levels and increased eosinophils infiltration, suggesting that TSLP production was responsible for the extent of Th2-biased inflammation, and the production of TSLP in the airway upon allergen exposure might be the early event in the immune cascade leading to subsequent allergic inflammation. Previous studies revealed that TSLP was mainly expressed by barrier epithelial cells and these cells produced large quantities of TSLP in response to certain microbial products including bacterial peptidoglycan (PGN), lipoteichoic acid (LTA) and poly I:C, as well as physical injury, providing the explanation for aggravating effect of some infection and trauma in asthma and atopic dermatitis [33,34]. More interestingly, expression of TSLP by airway epithelial cells or epidermal cells also showed to be triggered by proinflammatory cytokines TNF- α or IL-1 α , as well as Th2 cytokines IL-4 or IL-13, implying that a positive feedback through TSLP signaling might exist to maintain and amplify allergic inflammation [35,36]. These studies underscored the importance of the TSLP signaling in Th2-mediated inflammation, and raised the feasibility of developing TSLP/TSLPR-targeted treatments for allergic diseases [37]. However, as previous studies shown, a low level of TSLP was critical to maintain lymphocytes development and homeostasis, and systematic disruption of TSLP production was associated with some immune disorders like Crohn's disease [16,17]. Therefore, better understanding of the function and mechanism of TSLPR signaling in this inappropriate allergic inflammation and comprehensive assessment of the effects of TSLPR blockade on the development of allergic diseases are essential to design TSLP-oriented therapies.

Here, using OVA-driven asthmatic mice models, we demonstrated that local blocking TSLP signaling with i.t. application of anti-TSLPR substantially suppressed the airway inflammatory reactions, characterized with little infiltration of eosinophils and lymphocytes, minor mucus hyperplasia, and decreased production of Th2 cytokines. This finding established the critical role of TSLPR-mediated signaling in Th2 priming in asthma while ruling out the possible systematic effects derived from *tslpr* knockout, and more importantly, demonstrated the preventing effects of TSLPR inhibition in allergic airway inflammation. To further illuminate the underlying mechanism of anti-inflammatory effects of TSLPR inhibition, we focused on airway DCs, the critical players in asthmatic responses, also the cells preferentially expressing TSLPR [38]. We firstly assessed the maturation of lung DCs upon allergen encounter and found that DCs from mice with anti-TSLPR administration was less activated as demonstrated by reduced expression of CD40, CD80, CD86 and MHCII compared with those from the Ig-treated mice. Furthermore, a comparison of the numbers of OVA-loaded DCs in MLNs of different groups of mice showed that anti-TSLPR treatment caused a rapid reduction of OVA-loaded DCs migrating into the draining LNs, suggesting that blocking of TSLPR significantly inhibited the

migratory activity of airway DCs in response to allergen, which might be an important biological function mediated by TSLPR signaling in the development of allergic inflammation. This assumption was confirmed by the previous observation, which showed that increased TSLP induced by atopic dermatitis was closely associated with the migration of Langerhans cells into the draining lymph nodes to initiate allergic responses [7]. Since maturation and mobilization of airway DCs are prerequisites to prime allergic inflammation, down-regulation of these activities by TSLPR blocking might be responsible for the suppressed Th2 priming and inflammatory response observed in our experiments.

To further elucidate the role of TSLP signaling in DC-mediated Th2 priming, we evaluated the effects of TSLPR blocking on the capability of DCs to stimulate and polarize T cell response by coculturing BM-DCs with OVA-specific DO11.10 T cells. It was found that TSLPR blocking significantly impaired the capability of TSLP-conditioned DCs to drive antigen-specific T cell proliferation. More strikingly, anti-TSLPR administration appeared to remodel the DC-initiated TH1/2 response by down-regulating IL-4 levels while augmenting IFN- γ levels. This result was in accord with the cytokine profile we observed in the BALF of anti-TSLPR-treated asthmatic mice, and stressed the importance of TSLPR-mediated signaling in the conditioning of DCs towards a proallergic function. Also, blocking of TSLPR in total splenocyte culture led to a boosted production of IFN- γ , with a mild reduced IL-4 production though (data not shown), implicating IFN- γ in the effects of TSLPR inhibition in Th2-mediated allergic inflammation. However, IFN- γ didn't necessarily serve as Th1 cytokine in asthmatic pathogenesis, as some tantalizing data indicated that plasmacytoid DCs (pDCs), a specific DC subtype with intrinsic inhibiting property against harmless antigen [39], can rapidly induce IFN- γ production during sensitization, which may exert an immunoregulatory role to abate the inflammatory response. Additionally, a novel IFN- γ -producing regulatory T cells had been identified and displayed the capability of delivering preventing effects to allergic diseases [40,41]. From this perspective, the significance of IFN- γ in the mechanism of TSLPR-mediated biological activity needs further investigation, and it appears to be of great interest to investigate the possible involvement of anti-inflammatory regulatory T cells or pDCs in the preventing effects of TSLPR inhibition.

Our studies showed that modulation of DCs function by TSLPR inhibition accounted, at least partly, for the lessened severity of asthma in mice. However, more recent studies [42,43] demonstrated that TSLPR expressed not only on DCs, but also on mast cells, macrophages, and CD4⁺T cells. To rule out the direct effects of TSLPR neutralization on CD4⁺T cells or other effector cells in allergic inflammation, we further performed experiments in which OVA-loaded DCs were *in vitro* treated with anti-TSLPR prior to adoptive transfer to the airways of naive mice. As shown in previous reports [21,24], OVA-DCs triggered a strong eosinophilic airway inflammation, and accompanied with massive production of Th2 cytokines, such as IL-4 and IL-5. However, pretreating OVA-DCs with anti-TSLPR resulted in the significant reduction of eosinophils and lymphocytes infiltration, as well as IL-4 and IL-5 levels, further illuminating the putative role for TSLPR in DC-primed allergic disease, and demonstrating the potential of blocking TSLPR on DCs in controlling the airway inflammation.

In conclusion, our finding that local administration of anti-TSLPR inhibited Th2-mediated cardinal features of asthma by altering the function of lung DCs extended our understanding of biological function of TSLP/TSLPR and established the importance of TSLP receptors in atopic diseases, which is also supported by the observation of a strong link between atopic asthma and genetic polymorphisms in the human IL-7R α , a subunit shared with IL-7 and TSLP [44,45]. Therefore, it might be rewarding to determine whether polymorphisms in the TSLPR gene correlate with susceptibility to allergic inflammation, and further studies on the physiological and pathological significance of TSLP and TSLP receptor will shed more light on allergic pathogenesis and opens new therapeutic perspective in these diseases.

Conflict of interest

None of the authors has any potential financial conflict of interest related to this manuscript.

Acknowledgments

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