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## ORIGINAL ARTICLE

**Correlation of TSLP, IL-33, and CD4 + CD25 + FOXP3 + T regulatory (Treg) in pediatric asthma**

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**Abstract**

**Objective:** Newly discovered cytokines TSLP and IL33 are being studied as important indicators of Th2 inflammation and their effect on Treg cells is likely to modulate immune response. We attempted to study TSLP and IL-33 and then correlated with Tregs in order to find possible biomarker in these patients. **Methods:** Sixty-five children (37 with asthma only and 28 with asthma and rhinitis) aged  $6.4 \pm 3.2$  years (patient group) and 15 healthy children aged  $8.0 \pm 2.6$  years (control group) were recruited in this study. *In vitro* analysis of TSLP and IL-33 was done in serum samples of 65 newly diagnosed children for allergic asthma and 15 healthy children using the sandwich ELISA method. The expression of Treg cells (CD4 + CD25 + FOXP3+) was analyzed by flow cytometry. **Results:** The mean TSLP in the patient group ( $592 \pm 68$  pg/ml) was significantly higher than controls ( $215 \pm 45$  pg/ml) ( $p < 0.05$ ). Alternatively, the expression of FOXP3 + T reg cells was significantly lower in the patient group ( $52 \pm 36$ ) compared with the controls ( $95.9 \pm 3.6$ ) ( $p = 0.003$ ). IL-33 was also significantly higher ( $4044 \pm 413$  pg/ml) in the patient group compared with the controls ( $3282 \pm 331.5$  pg/ml) ( $p = 0.0001$ ). The expression of Treg cells was negatively correlated with the TSLP ( $r = -0.23$ ,  $p = 0.07$ ). Asthma control test (ACT) was also negatively correlated with TSLP in the patient group ( $r = -0.14$ ,  $p > 0.05$ ). **Conclusion:** Children with asthma show elevated serum levels of TSLP, which correlated negatively with asthma control test and Treg cells. TSLP may be used as a biomarker for inflammation in pediatric asthma patients.

**Keywords**

ACT, allergic inflammation, allergy, FEV1, pulmonary function test

**History**

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**Introduction**

Asthma is a chronic inflammatory disorder of the airways characterized by a predominant Th2 response. TSLP has been considered to switch on the Th2 response in asthma. TSLP is a distant paralog of IL-7 and a member of the interleukin IL-2 cytokine family [1]. Like IL-7, TSLP can stimulate thymocytes and promote B-cell lymphopoiesis [2]. The primary producers of TSLP in allergic inflammation are epithelial cells, keratinocytes, and stromal cells, although recent data have demonstrated that both dendritic cells (DCs) and mast cells are also capable of TSLP production [3–7]. TSLP directly and selectively impairs IL-10 production of Treg cells and inhibited their suppressive activity [8]. Thymic stromal lymphopoietin overproduced by keratinocytes in mouse skin aggravates experimental asthma [9]. The increased level of TSLP is found in lung biopsies of asthma patients, which is implicated in the pathogenesis of airway disease [5]. Similarly, IL-33, a member of the IL-1 cytokine family,

serves as an alarm in pro-allergic inflammatory cytokine as well as a transcriptional regulator. In severe asthma patients, IL-33 expression is increased [10]. Further, IL-33 can induce TSLP production from epithelial cells [11]. IL-33 might act as a link between cells in different tissue compartments to regulate the adaptive immune response as it is produced in a fully active form by airway epithelial cells, fibroblasts, and smooth muscle cells. The central role of IL-33 is in driving Th2-mediated inflammation, in human subjects and mouse models of allergic airway disease. Cytokines, such as IL-5 and IL-13, are produced from a novel population of IL-33-responsive innate immune cells, the type 2 innate helper cells [12]. TSLP and IL-33 are secreted by the airway epithelium that regulates immune-mediated inflammatory airway diseases and the response to infections. The genes for IL-33 and TSLP have emerged as two of the most important associations for the development of the asthma [13]. T reg cells are key regulators of inflammatory responses [14,15]. There is decreased frequency and diminished suppressive activity of T reg cells in human asthma [16]. TSLP has been seen to influence the development of these T reg cells [17]. There is disruption of TSLP signaling after TSLP receptor is deleted, impairing intra-thymic generation of T reg cells but did not affect their peripheral repertoire [18]. TSLP and IL-33 might contribute to the exacerbation of airway inflammation by

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directly inhibiting tolerogenic immune responses of pulmonary T reg [8]. Until now, there exists no study on direct correlation among TSLP, IL-33, and T reg cells in pediatric asthma. Thus, the present study was designed to determine the role of TSLP, IL-33, and T reg cells and interplay among these in an attempt to find a possible biomarker for allergic inflammation in pediatric asthma.

Method

Sixty-five newly diagnosed children with asthma with or without allergic rhinitis (1–12 years age) were enrolled after obtaining their informed written consent from their parents/guardians in the study from Allergy and Asthma Clinic of Advanced Pediatrics Centre, Post-Graduate Institute of Medical Education and Research PGIMER, Chandigarh. The diagnosis of asthma was made according to the Global Initiative for Asthma (GINA) guidelines. The details of clinical history, co-morbid conditions, anthropometric measurement, and lung spirometry were recorded on patient performa. The patient and control groups were matched. We took 15 healthy children belong to the same age group. Severity and control of asthma symptoms was detected by clinical examination, spirometry (where possible) and by asthma control test. Asthma Control Test was used to measure control of asthma patients. ACT is a quick test consisting of five questions which provides a numerical score to help assess asthma control. A score of 19 or less, predicts asthma as uncontrolled [19]. Spirometry was carried out in the patient and the control group. FEV1% of the children was assessed by using a spirolab II (Medical International Research SN, Agra, India, A23-050.0814) [20]. The study was approved by the Institute’s Ethic Committee of PGIMER, Chandigarh.

Estimation of TSLP and IL-33

Plasma samples of patients and control were analyzed for TSLP using sandwich ELISA kit (Ray Bio Human TSLP ELISA kit, RayBiotech, Inc., Norcross, GA) and IL-33 was analyzed by Development ELISA kit (PeproTech Human Mini ELISA Development kit, PeproTech, Inc., Rocky Hill, NJ).

CD4 + CD25 + T reg cells were estimated using flow cytometry. Peripheral blood mononuclear cells (PBMC) isolated from peripheral blood were screened for CD4 + CD25+ cells, which were further analyzed for FOXP3+ Treg cell expression. Permeabilization and fixation were carried out with para-formaldehyde, according to the instruction manufacturer (BD Biosciences, San Diego, CA). Analysis was done by FACS Canto (Becton Dickinson, Mountain View, CA) with the FACS Diva TM Software (Becton Dickinson, Mountain View, CA). The following maps (BD Biosciences, San Diego, CA) were used: APC anti-human CD4, PE-Cy TM anti-human CD25, and PE anti-human FOXP3.

Statistical analysis

Data were analyzed with Graph pad prism (v6.2; GraphPad Software Inc., Le Jolla, CA) and STATA 12 (Stata Corp LP, College Station, TX). The data were checked for the normalcy of distribution. Mean and SD were calculated for continuous variables and proportions were calculated for categorical variables. The flow cytometric data analyzed by Student’s *t*-test and Spearman’s rank correlation coefficient were used to evaluate the relationship between variables. Statistical significance was considered for *p* < 0.05.

Result

Sixty-five children (37 with asthma only and 28 with asthma and rhinitis) within the age group of 6.4 ± 3.2 years (patient group) and 15 healthy children within the age group 8.0 ± 2.6 years (control group) were recruited after taking written informed consent from parents/guardians of all included patients and the control group. Three of the 28 children with asthma and rhinitis were having rhinosinusitis. There was no significant difference in age and weight, between the patient and the control group (*p* > 0.05). However, BMI was higher in the control group as compared with the patient group (*p* = 0.05, Table 1). There is a significant difference in FEV1% between the patient (56 ± 41) % and the control group (95 ± 16)% (*p* = 0.0015) (Table 2). Out of the total enrolled

Table 1. Demographic and anthropometric parameters of the patient and the control group.

| Parameters               | Patient, <i>n</i> = 65           | Control, <i>n</i> = 15            | <i>p</i> Value |
|--------------------------|----------------------------------|-----------------------------------|----------------|
| Age (years)              | 6.4 ± 3.2 (95% CI 5.9–7.4)       | 8.0 ± 2.6 95% CI (7.7–10.7)       | >0.05          |
| Male ( <i>n</i> ; %)     | 52                               | 11                                | <0.05          |
| Weight (kg)              | 21.27 ± 9.2 (95% CI 19–23.02)    | 26.17 ± 11.0 95% CI (20.0–32.4)   | >0.05          |
| Height (cm)              | 112 ± 26.7 (95% CI 106–119)      | 130.3 ± 22 95% CI (119.5–141.0)   | 0.002          |
| BMI (kg/m <sup>2</sup> ) | 15.49 ± 2.84 (95% CI 14.8–16.17) | 16.88 ± 3.059 95% CI (15.4–18.35) | 0.05           |

Table 2. Compared value of FEV1%, TSLP, IL-33, and FOXP3 + Treg cells between patient and control group.

| Parameters                       | Patient, <i>n</i> = 65            | Control, <i>n</i> = 15          | <i>p</i> Value |
|----------------------------------|-----------------------------------|---------------------------------|----------------|
| ACT                              | 20.05 ± 2.58 (95% CI 19.31–20.48) | –                               | –              |
| FEV1 % ( <i>n</i> = 50)          | 56 ± 41 (95% CI 44.5–73.1)        | 95 ± 16 95% CI (87.1–104.5)     | 0.0007         |
| TSLP pg/ml                       | 592 ± 68 (95% CI 457–728)         | 215 ± 45 (95% CI 111.7–319.7)   | 0.041          |
| IL-33 pg/ml                      | 4044 ± 413 (95% CI 3955–4151)     | 3282 ± 331.5 (95% CI 3188–3347) | 0.0001         |
| %CD4 + CD25 + FOXP3 + Treg cells | 52 ± 36 (95% CI 42.4–61.7)        | 95.9 ± 3.6 95% CI (94.1–97.2)   | 0.0003         |

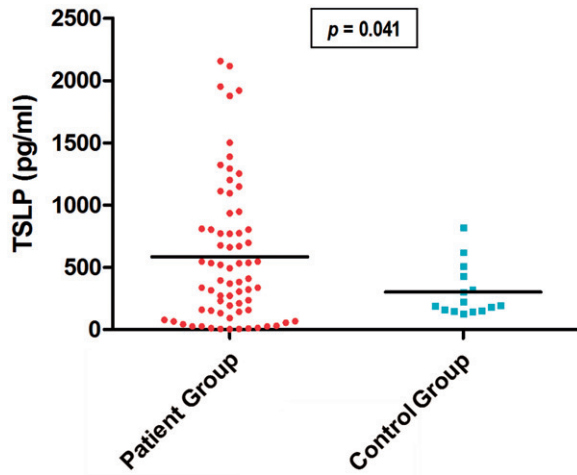


Figure 1. Comparison of TSLP levels in the patient and the control group.

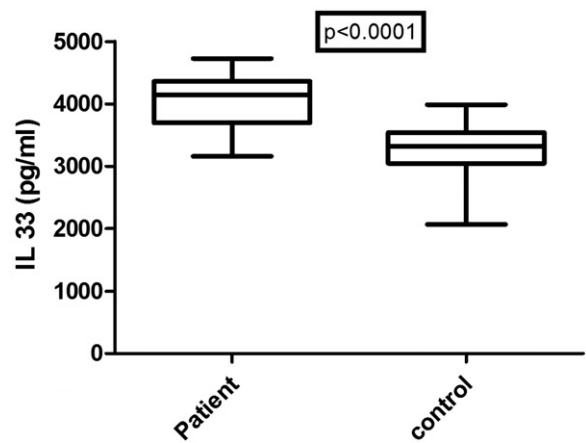


Figure 3. The level of IL-33 in the patient and the control group.

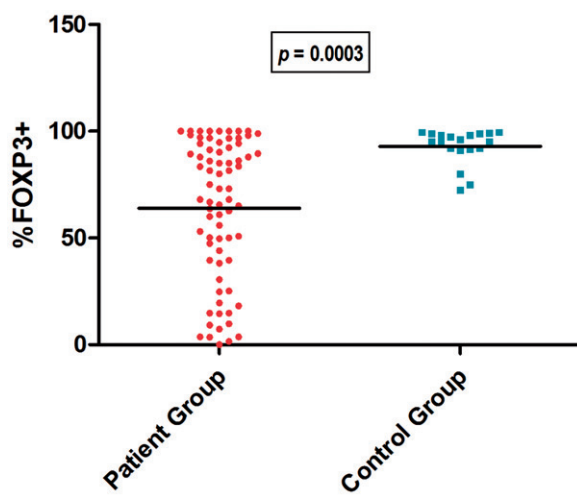


Figure 2. %FOXP3 expression in the patient and the control group.

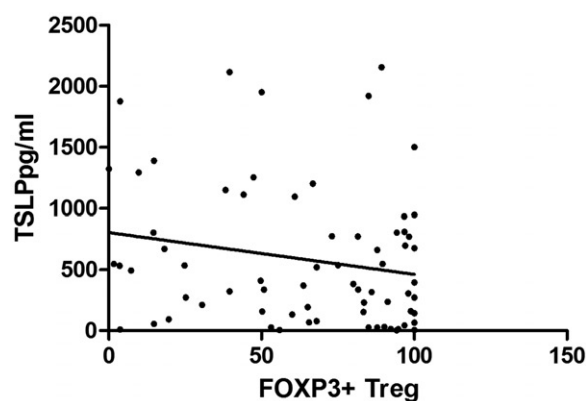


Figure 4. Correlation of TSLP and FOXP3+ in the patient group.

patients, 73.0% ( $n=48$ ) of the patients were on inhaled glucocorticoid therapy, Budesonide 400  $\mu\text{g}/\text{d}$ ; at the time of presentation, 10 patients were not on any medications and the remaining five were taking Fluticasone of 200  $\mu\text{g}/\text{d}$ , three patients were taking Fluticasone 200  $\mu\text{g}$  with Salmeterol 50  $\mu\text{g}$  twice a day. None of the children were taking anti-leukotrienes at the time of presentation included in the study.

The mean level of TSLP in the patient group ( $592 \pm 68$  pg/ml) was significantly higher compared with control group ( $215 \pm 45$  pg/ml) ( $p < 0.05$ ; Table 2 and Figure 1). Alternatively the expression of FOXP3+ T reg cells were significantly lower in the patient group ( $52 \pm 36$ )% as compared with the control group ( $95.9 \pm 3.6$ )% ( $p = 0.003$ ; Figure 2). IL-33 was also significantly higher ( $4044 \pm 413$  pg/ml) in the patient group compared with the control group ( $3282 \pm 331.5$  pg/ml) ( $p = 0.0001$ ; Table 2 and Figure 3). Expression of FOXP3+ negatively correlated with the TSLP levels in the patient group ( $r = -0.23$ ,  $p = 0.07$ ; Figure 4). There was a weak negative correlation between TSLP and ACT ( $r = -0.14$ ,  $p > 0.05$ ). The representative plot of CD4+ CD25+ FOXP3+ T regulatory cell in the patient and control group is demonstrated (Figure 5).

### Discussion

In our study, we have found higher level of TSLP in children with asthma responsible for their airway inflammatory disease. The results of our study are similar with the previous study done in epithelial cells where the increased levels are found in children with asthma [5]. The present study demonstrates higher level of IL-33 and TSLP in children with asthma as compared with the control group. IL-33 can induce TSLP production from epithelial cells [12]. These two cytokines TSLP and IL-33 are the major switches of Th2 polarization. FOXP3+ Treg cell expression was decreased in the patient group as compared with the control group. TSLP and IL-33 were negatively correlated with the measured FOXP3+ Treg expression in these patients. Subsequently, ACT was done to determine their asthma control scores. ACT was also negatively correlated with the TSLP expression in these patients.

Recent studies have identified the airway epithelium as a major source of IL-33 and TSLP that regulates immune-mediated inflammatory airway diseases and the response to infections [12]. In a murine model, TSLP has been identified as a “master switch” for allergic inflammation [21]. Studies on TSLP polymorphism demonstrate association with childhood and adulthood allergic asthma [22,23]. TSLP also induces the production of Th2 cytokines from human mast

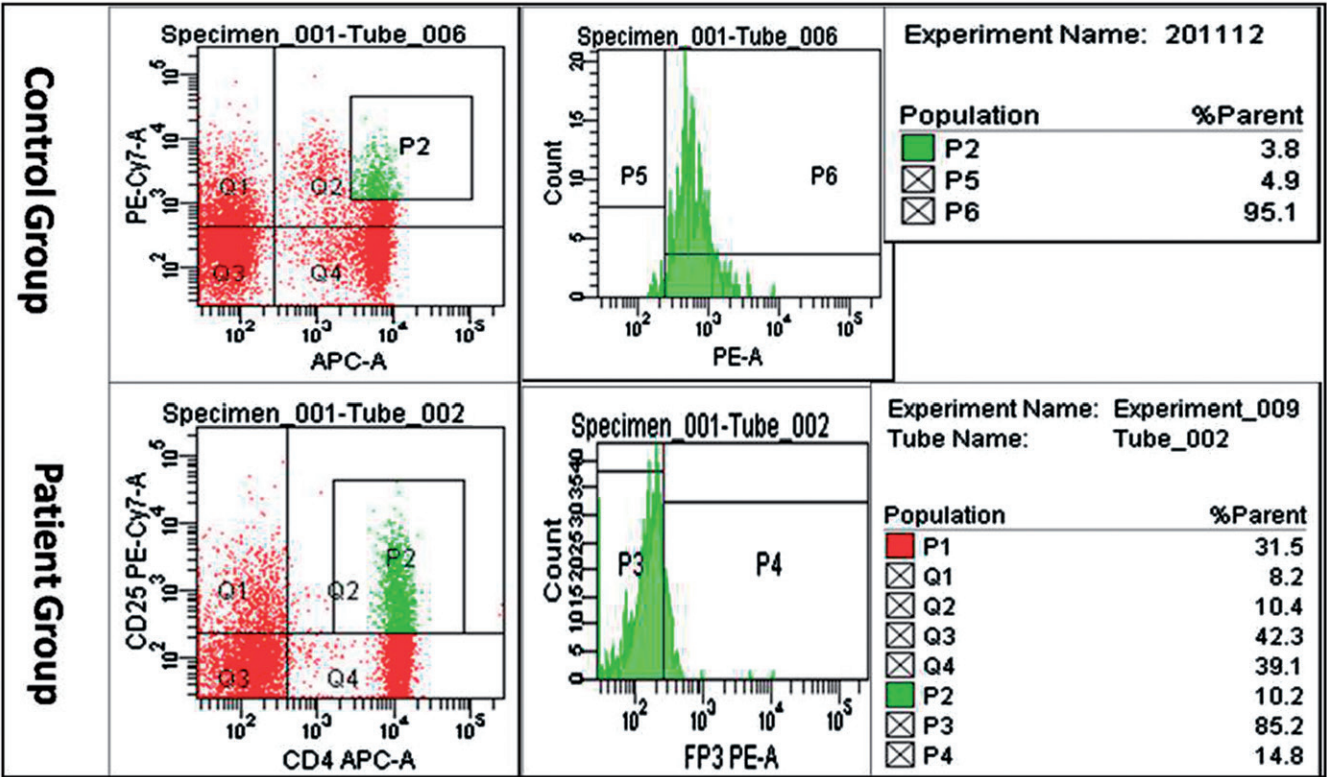


Figure 5. The expression level of CD4<sup>+</sup>CD25<sup>+</sup> FOXP3<sup>+</sup> was examined by flow cytometry in the control and the patient group.

cells [24]. Further, IL-33 is produced in a fully active form by airway epithelial cells, fibroblasts, and smooth muscle cells and, therefore, might also act as a link between cells in different tissue compartments to regulate the adaptive immune response [11]. The immune and inflammatory processes that underpin asthmatic disease are associated with impaired immune regulation by both FOXP3<sup>+</sup> and IL-10 T regulatory (Treg) cells [25]. Further, studies on human asthma have demonstrated decreased frequency of pulmonary Tregs and their diminished suppressive activity [16]. Our study is an association study demonstrating decreased levels of %FOXP3<sup>+</sup> Treg cells and negative correlation with TSLP. This is consistent with the results of a study which demonstrates a direct role of TSLP in the regulation of suppressive activities of T reg [8]. The features of asthma in a murine model, including airway inflammation, infiltration by eosinophils, goblet cell hyperplasia and AHR, are induced by lung-specific transgenic expression of TSLP [26,27]. There is no development of eosinophilic airway inflammation in mice lacking the TSLP receptor (TSLPR) after challenging with experimental allergen [28].

Taking into account of all the parameters, TSLP can be one of the potentially promising biomarkers in children with asthma. In a recent study showing effects of an anti-TSLP antibody on allergen-induced asthmatic responses, TSLP was targeted with AMG157 (700 mg) which resulted in reducing allergen-induced bronchoconstriction [29]. Our study thus supports TSLP, as an effective cytokine which could be targeted with specific therapy. Asthma control was also reduced in comparison with the increased level of TSLP in children with asthma. Further larger clinical trials are needed to target TSLP and IL-33 with immunotherapy.

**Conclusion**

Children with asthma show elevated serum levels of TSLP, which correlated negatively with asthma control test and T reg cells. TSLP may be used as a biomarker for inflammation in pediatric asthma patients.

**Declaration of interest**

The authors report that they have no conflicts of interest to disclose. The authors thank Indian Council of Medical Research, New Delhi, for the support.

**References**

- Leonard WJ. TSLP: finally in the limelight. *Nat Immunol* 2002;3: 605–607.
- Levin SD, Koelling RM, Friend SL, Isaksen DE, Ziegler SF, Perlmutter RM, Farr AG. Thymic stromal lymphopoietin: a cytokine that promotes the development of IgM<sup>+</sup> B cells in vitro and signals via a novel mechanism. *J Immunol* 1999;162:677–683.
- Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3: 673–680.
- Watanabe N, Hanabuchi S, Soumelis V, Yuan W, Ho S, de Waal Malefyt R, Liu YJ. Human thymic stromal lymphopoietin promotes dendritic cell-mediated CD4<sup>+</sup> T cell homeostatic expansion. *Nat Immunol* 2004;5:426–434.
- Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, Robinson D, et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 2005; 174:8183–8190.
- Kashyap M, Rochman Y, Spolski R, Samsel L, Leonard WJ. Thymic stromal lymphopoietin is produced by dendritic cells. *J Immunol* 2011;187:1207–1211.

7. Moon PD, Kim HM. Thymic stromal lymphopoietin is expressed and produced by caspase-1/NF-kappaB pathway in mast cells. *Cytokine* 2011;54:239–243.
8. Nguyen KD, Vanichsarn C, Nadeau KC. TSLP directly impairs pulmonary Treg function: association with aberrant tolerogenic immunity in asthmatic airway. *Allergy Asthma Clin Immunol* 2010;6:4 doi: 10.1186/1710-1492-6-4.
9. Zhang Z, Hener P, Frossard N, Kato S, Metzger D, Li M, Chambon P. Thymic stromal lymphopoietin overproduced by keratinocytes in mouse skin aggravates experimental asthma. *Proc Natl Acad Sci USA* 2009;106:1536–1541.
10. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, Martin JG, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;125:752–754.
11. Saenz SA, Taylor BC, Artis D. Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. *Immunol Rev* 2008;226:172–190.
12. Licona-Limon P, Kim LK, Palm NW, Flavell RA. TH2, allergy and group 2 innate lymphoid cells. *Nat Immunol* 2013;14:536–542.
13. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von Mutius E, et al. A large-scale, consortium-based genome-wide association study of asthma. *N Engl J Med* 2010;363:1211–1221.
14. Akdis CA, Akdis M. Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. *J Allergy Clin Immunol* 2009;123:735–746.
15. Herrick CA, Bottomly K. To respond or not to respond: T cells in allergic asthma. *Nat Rev Immunol* 2003;3:405–412.
16. Hartl D, Koller B, Mehlhorn AT, Reinhardt D, Nicolai T, Schendel DJ, Griesse M, et al. Quantitative and functional impairment of pulmonary CD4<sup>+</sup>CD25<sup>hi</sup> regulatory T cells in pediatric asthma. *J Allergy Clin Immunol* 2007;119:1258–1266.
17. Watanabe N, Wang YH, Lee HK, Ito T, Wang YH, Cao W, Liu YJ. Hassall's corpuscles instruct dendritic cells to induce CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in human thymus. *Nature* 2005;436:1181–1185.
18. Jiang Q, Coffield VM, Kondo M, Su L. TSLP is involved in expansion of early thymocyte progenitors. *BMC Immunol* 2007;8:11.
19. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, Murray JJ, Pendergraft TB. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;113:59–66.
20. Laszlo G. ATS/ERS task force: standardisation of lung function testing. *Eur Respir J* 2005;26:319–338.
21. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144–2148.
22. Harada M, Hirota T, Jodo AI, Hitomi Y, Sakashita M, Tsunoda T, Miyagawa T, et al. Thymic stromal lymphopoietin gene promoter polymorphisms are associated with susceptibility to bronchial asthma. *Am J Respir Cell Mol Biol* 2011;44:787–793.
23. Bunyavanich S, Melen E, Wilk JB, Granada M, Soto-Quiros ME, Avila L, Lasky-Su J, et al. Thymic stromal lymphopoietin (TSLP) is associated with allergic rhinitis in children with asthma. *Clin Mol Allergy* 2011;9:1 doi: 10.1186/1476-7961-9-1.
24. Allakhverdi Z, Comeau MR, Jessup HK, Yoon BR, Brewer A, Chartier S, Paquette N, et al. Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J Exp Med* 2007;204:253–258.
25. Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. *Immunity* 2009;31:438–449.
26. Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard WJ. A role for TSLP in the development of inflammation in an asthma model. *J Exp Med* 2005;202:829–839.
27. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, Gyarmati D, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;6:1047–1053.
28. Kato A, Favoreto Jr S, Avila PC, Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J Immunol* 2007;179:1080–1087.
29. Gauvreau GM, O'Byrne PM, Boulet LP, Wang Y, Cockcroft D, Bigler J, FitzGerald JM, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med* 2014;370:2102–2110.