

Review Article

Thymic Stromal Lymphopoietin (TSLP) as a Bridge between Infection and Atopy

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Received 12 September 2007; Accepted 20 October 2007; Available online 1 January 2008

Abstract: The rising worldwide prevalence of asthma has intensified interest in the natural history of asthma. An improved understanding of the genetic, environmental, and developmental factors contributing to the inception and exacerbation of asthma will be crucial to efforts to devise effective preventive and therapeutic interventions. There is increasing evidence that the complex interplay of early life respiratory viral infections and allergic sensitization is important in the development of asthma. Major causes of asthma exacerbations are respiratory viral infections and aeroallergen exposure, which may have interactive co-morbid effects. This review describes the potential role of thymic stromal lymphopoietin (TSLP) as a connection between the innate immune response to respiratory viral infections and the type-2 adaptive immune response in the development and exacerbation of asthma.

Key Words: Thymic stromal lymphopoietin (TSLP), virus infection, atopy, asthma

Introduction

There is compelling evidence that Th-2 lymphocytes regulate the immune response responsible for allergy and asthma. Their products, so called type-2 cytokines, such as interleukin 4 (IL-4), IL-5, IL-9, and IL-13 are upregulated in the blood, bronchoalveolar lavage (BAL) and airway biopsies from allergic and asthmatic patients with active disease [1-4]. Th-2 cytokines released by mast cells during the early-phase reaction [5] or memory CD4+ Th-2 lymphocytes [6] are likely triggers for the subsequent recruitment of eosinophils, which characterize the chronic phase of asthma. However, the cause of Th-2 immune responses and asthma development remain thinly recognized.

Respiratory virus infections have been associated with the inception and exacerbation of asthma [7-11]. The pathology of a viral infection is complex and includes epithelial barrier damage, with increased environmental toxin penetration, and the

accumulation and activation of inflammatory cells in the airways [12].

Thymic stromal lymphopoietin (TSLP) was originally identified in conditioned medium from a thymic stromal cell line (Z210R.1) and showed activity as a B cell growth factor [13]. TSLP interacts with the IL-7R α -chain and displays overlapping effects with IL-7 on B220+/IgM+ immature B cells [13]. Unlike IL-7, TSLP does not activate Janus family kinases (JAKs) probably due to signaling through a specific TSLP co-receptor named TSLPR or CRLF2 [14]. TSLP increases the proliferation of murine CD4-, CD8- double negative thymocytes in synergy with IL-1 [15]. Recently, TSLP has been proposed as a liaison between the viral-triggered innate response and the type-2 adaptive immune response and eosinophilic inflammation.

Asthma and Viral Infection

Respiratory virus infections in early life have been associated with the development of

persistent wheezing and childhood asthma and are the major cause of asthma exacerbations in children and adults. Respiratory syncytial virus (RSV)-induced bronchiolitis has been shown to lead to persistent wheezing [16] as well the development of asthma in young children [17]. More recently, rhinovirus infections have been strongly associated with the development of persistent wheezing in childhood [18, 19]. Multiple studies have shown that close to 80% of asthma exacerbations in children and adults were preceded by respiratory virus infection, with the majority due to rhinoviruses [20-23]. Viral infection may correlate with asthma exacerbations because of the development of the type-2 immune response in asthmatic individuals, leading to reduced IFN- γ and IL-12, and inefficient antiviral immunity in these individuals. Viruses can exert profound effects on epithelial cells, which may redirect the immune system toward a type-2 response with the production of IgE. For example, human rhinovirus (HRV) triggers nasal and sputum IL-6 production [24]. IL-6 can facilitate differentiation of B-cells towards IgE-positive mature B cells [25]. However, IL-6 is present at similar levels after HRV infection in both non-atopic and asthmatic individuals. However, airway epithelial cells from asthmatic patients are deficient in the generation of interferon- β (IFN- β) and IFN- γ after infection with HRV [26, 27]. HRV also upregulates the expression of multiples chemokines, including RANTES (CCL5) [28], which is a powerful attractant for eosinophils [29]. Under these conditions, the expression of inducible nitric oxide synthase (iNOS) and nitric oxide (NO) are increased [30]. NO is associated with eosinophilia, airway inflammation [31] and with type-2 differentiation [32].

Virus and TSLP

Epithelial cells, lung fibroblasts, mast cells, keratinocytes and smooth muscle cells produce TSLP [33]. TSLP is minimally expressed by endothelial or hematopoietic cells, with the exception of mast cells activated by IgE receptor cross-linking. While epithelial cells and lung fibroblasts constitutively express substantial TSLP, bronchial smooth muscle and skin keratinocytes express TSLP only after activation with multiple cytokines (IL-4, IL13 and TNF- α or TNF- α and IL-1 β) [33].

Keratinocyte growth factor (KGF), expressed by thymocytes, induces cortical and medullary thymic epithelial cells [34] to produce TSLP but not IL-7 [34]. The nuclear receptor agonist, vitamin D3, also induces TSLP expression in epidermal keratinocytes. In contrast, keratinocytes from nuclear receptor (retinoid X receptor α and β) knockout mice express high levels of TSLP [35]. Small airway epithelial cells (SAECs) stimulated with inflammatory cytokines (IL-1 plus TNF- α), bacterial peptidoglycan (PGN) and toll-like receptor (TLR1, 2 and 3) ligands, such as lipoteichoic acid (LTA) from *Bacillus subtilis* or poly I:C (double-stranded RNA), produce TSLP [36]. On note, infection with the bacteria *S. thyphimurium* also increases TSLP expression in Caco-2 cells [37], suggesting that bacterial as well as viral infections can induce TSLP. IL-4 or IL-13 induce up-regulation of TSLP mRNA by human bronchial epithelial cells [38]. However, poly I:C double stranded RNA (dsRNA), a ligand for TLR3, is the most potent inducer of TSLP mRNA. DsRNA acts synergistically with IL-4 in TSLP induction. None of the other TLR ligands (TLR2, 4, 5, 6, 7 or 9) affected TSLP mRNA or protein production [38]. Rhinoviruses synthesize dsRNA during replication, and they are a natural source of TLR3 ligand. RV16 in association with IL-4, strongly induces TSLP production by epithelial cells in a manner that is dependent on TLR3, NF-kappaB and IRF-3 [38]. TSLP mRNA can also accumulate in epithelial cells after exposure to the pro-inflammatory cytokines IL-1 β or TNF- α or to TLR2, 8 and 9 ligands [39]. Both sets of activators trigger nuclear translocation, which likely participates in TSLP transcriptional regulation. Therefore, viral infection in synergy with cytokine production is likely to be a primary driver for TSLP production.

TSLP and Asthma

TSLP upregulates activation markers (HLA-DR, CD40, CD80, CD86 and CD83) and prolongs survival of CD11c+ dendritic cells (DCs) [33]. TSLP-activated DCs induce stronger allogeneic CD4+ cell proliferation than DC activated with CD40 ligand or IL-7. TSLP-activated DCs do not produce proinflammatory cytokines but large amounts of chemokines TARC and MDC, which attract CCR4-expressing Th-2 cells [33]. Strikingly, TSLP-activated DCs induce naïve CD4+ T lymphocytes to produce substantial IL-

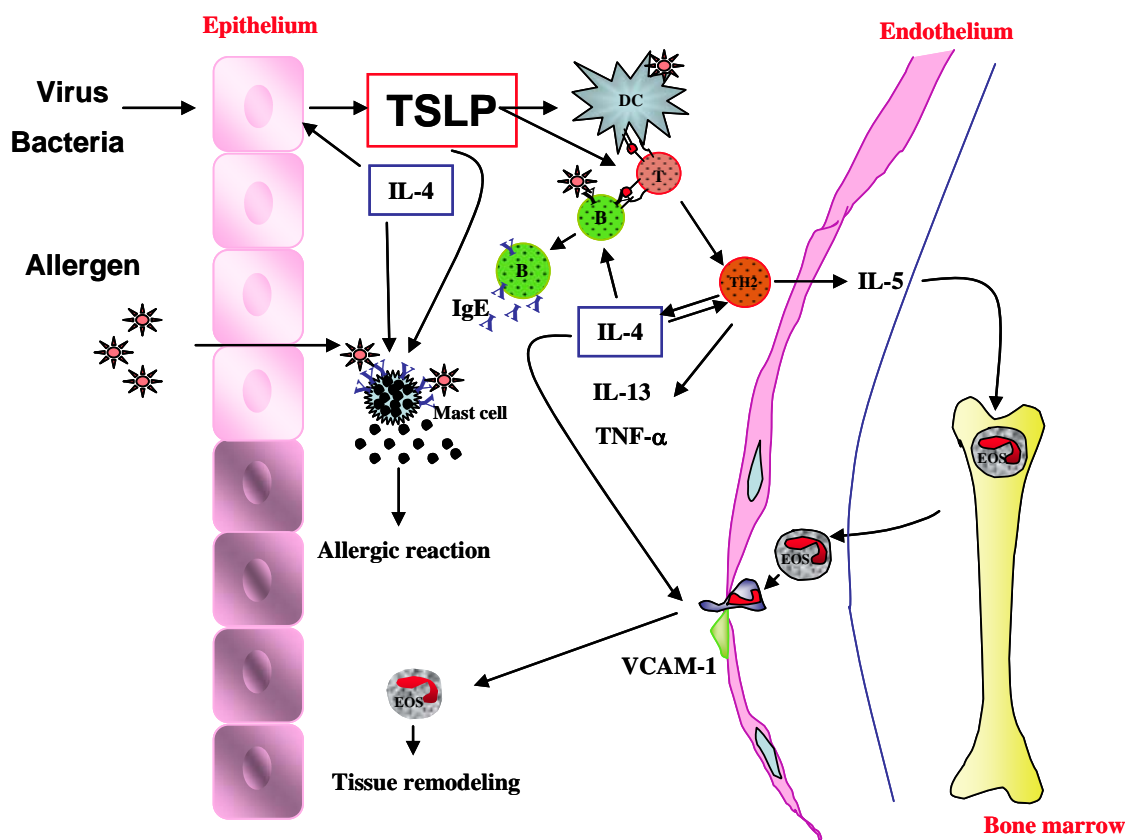


Figure 1 Viral infection leads to atopy through TSLP. Virus or bacteria activate epithelial cells to produce TSLP, which induces a type-2 lymphocyte (TH2) response with production of IL-4, IL-5, IL-13 and TNF- α . TSLP may as well direct the allergen-activated dendritic cells (DC) to trigger a TH2 response after DC-T lymphocyte interactions. Among the type 2 cytokines, IL-4 participates in the formation of IgE-producing B lymphocytes (B) and the recruitment of eosinophils (EOS) and, with TSLP, the activation of mast cells. In addition, IL-4, in association with viruses, activates epithelial cells to produce more TSLP. IL-5 induces differentiation of EOS, which accumulate at the site of inflammation, culminating in tissue remodeling.

4, IL-13, IL-5 and TNF- α but minimal IFN- γ and IL-10. TSLP is highly expressed in several type 2 inflammatory models and by keratinocytes, and TSLP expression correlates with DC activation in the dermis in atopic dermatitis [33]. The response of resident dermal Langerhans cells to TSLP is very similar to that of blood DCs [40].

The lungs of mice sensitized and challenged with ovalbumin (OVA) express high levels of TSLP [41]. Because these mice develop a type-2 immune response with increased serum IgE, infiltration of type 2 CD4+ cells, lung eosinophilia, and airway remodeling, these results suggest that TSLP participates in allergic inflammation. Several studies have confirmed this hypothesis. Forced pulmonary

expression of TSLP enhances BAL cell numbers, which consist primarily of eosinophils [41]. In these mice, infiltrating CD4+ T cells have a type-2 phenotype, and the lungs show characteristic remodeling, including epithelial cell hyperplasia, subepithelial fibrosis and mucus metaplasia [41]. The converse is seen in TSLP receptor knockout mice, which show reduced total BAL cells, eosinophils, and neutrophils as well as goblet cell numbers after OVA challenge [42]. TSLPR knockout mice have also significantly lower levels of serum IgE than do wild-type mice. Finally, decreased pulmonary inflammation occurs after neutralizing anti-TSLPR administration [42].

TSLP may broadly regulate systemic atopy.

TSLP transgenic mice develop atopic dermatitis as well as exaggerated levels of IgE, upregulation of E- and P-selectin ligands, and CCR4 [43]. In addition, the retinoid X receptor knockout mice, which express high level of TSLP, exhibit an atopic dermatitis-like phenotype [35].

TSLP not only plays a liaison role between the innate and adaptive immune response, but there is evidence that it may directly activate mast cells (MC) [36], which have a pivotal role during allergy [31]. Low level TSLP induces IL-13 and IL-5 production by MC [36]. A recent study demonstrated that not only human CD11c+ DC [44] but human CD4+ lymphocytes express TSLP receptor [45]. Although freshly isolated human B and T lymphocytes do not express TSLPR, activation *in vitro* leads to TSLPR expression, which persists for 14 days [45]. TSLP then induces Stat5 activation [46], and increases the proliferation of anti-CD3 or IL-2-activated human CD4+ T cells [45].

Conclusion

TSLP is an excellent candidate to mediate a link between the innate immune response triggered by viruses or bacteria and the subsequent adaptive response leading to atopic diseases, including atopic asthma (Figure 1). The mechanisms, which link allergen challenge and production of TSLP remain unknown. Further studies to better understand TSLP regulation, induction and function will likely shed additional light on this important cytokine.

Acknowledgements

The authors thank the financial support from NIH Grant AG25722 to DSW, AT002742 and AI070503 to LAR, and P50HL56396 to JSM.

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References

[1] Esnault S, Benbernou N, Lavaud F, Shin HC, Potron G and Guenounou M. Differential spontaneous expression of mRNA for IL-4, IL-10, IL-13, IL-2 and interferon-gamma (IFN-

gamma) in peripheral blood mononuclear cells (PBMC) from atopic patients. *Clin Exp Immunol* 1996;103:111-118.

- [2] Robinson DS, Hamid Q, Ying S, Tscopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR and Kay AB. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
- [3] Del Prete GF, De Carli M, D'Elisio MM, Maestrelli P, Ricci M, Fabbri L and Romagnani S. Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. *Eur J Immunol* 1993;23:1445-1449.
- [4] Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, Kay AB and Hamid QA. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;148:2390-2394
- [5] Turner H and Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature* 1999;402(6760 Suppl):B24-30.
- [6] Robinson DS, Bentley AM, Hartnell A, Kay AB and Durham SR. Activated memory T helper cells in bronchoalveolar lavage fluid from patients with atopic asthma: relation to asthma symptoms, lung function, and bronchial responsiveness. *Thorax* 1993;48:26-32.
- [7] Gern JE, Rosenthal LA, Sorkness RL and Lemanske Jr RF. Effects of viral respiratory infections on lung development and childhood asthma. *J Allergy Clin Immunol* 2005;115:668-674.
- [8] Lemanske Jr RF. Viral infections and asthma inception. *J Allergy Clin Immunol* 2004;114:1023-1026.
- [9] Friedlander SL, Jackson DJ, Gangnon RE, Evans MD, Li Z, Roberg KA, Anderson EL, Carlson-Dakes KT, Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ, Pleiss LE, Mikus LD, Rosenthal LA, Shult PA, Kirk CJ, Reisdorf E, Hoffjan S, Gern JE and Lemanske Jr RF. Viral infections, cytokine dysregulation and the origins of childhood asthma and allergic diseases. *Pediatr Infect Dis J* 2005;24:170-176.
- [10] Friedlander SL, Busse WW. The role of rhinovirus in asthma exacerbations. *J Allergy Clin Immunol* 2005;116:267-273.
- [11] Johnston SL. Innate immunity in the pathogenesis of virus-induced asthma exacerbations. *Proc Am Thorac Soc* 2007;4:267-270.
- [12] Gern JE and Busse WW. Relationship of viral infections to wheezing illnesses and asthma. *Nat Rev Immunol* 2002;2:132-138.
- [13] Friend SL, Hosier S, Nelson A, Foxworthe D, Williams DE and Farr A. A thymic stromal cell line supports *in vitro* development of surface IgM+ B cells and produces a novel growth

- factor affecting B and T lineage cells. *Exp Hematol* 1994;22:321-328.
- [14] Tonozuka Y, Fujio K, Sugiyama T, Nosaka T, Hirai M, Kitamura T. Molecular cloning of a human novel type I cytokine receptor related to delta1/TSLPR. *Cytogenet Cell Genet* 2001;93:23-25.
- [15] Sims JE, Williams DE, Morrissey PJ, Garka K, Foxworthe D, Price V, Friend SL, Farr A, Bedell MA, Jenkins NA, Copeland NG, Grabstein K and Paxton RJ. Molecular cloning and biological characterization of a novel murine lymphoid growth factor. *J Exp Med* 2000;192:671-680.
- [16] Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL and Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;354:541-545.
- [17] Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F and Kjellman B. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med* 2005;171:137-141.
- [18] Lemanske RF Jr, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, Carlson-Dakes KT, Adler KJ, Gilbertson-White S, Pappas TE, Dasilva DF, Tisler CJ and Gern JE. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005;116:571-577.
- [19] Kusel MM, de Klerk NH, Kebabdz T, Vohma V, Holt PG, Johnston SL and Sly PD. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007;119:1105-1110.
- [20] Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, Symington P, O'Toole S, Myint SH and Tyrrell DA, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 1995;310:1225-1229.
- [21] Kling S, Donninger H, Williams Z, Vermeulen J, Weinberg E, Latiff K, Ghildyal R, Bardin P. Persistence of rhinovirus RNA after asthma exacerbation in children. *Clin Exp Allergy* 2005;35:672-678.
- [22] Grissell TV, Powell H, Shafren DR, Boyle MJ, Hensley MJ, Jones PD, Whitehead BF and Gibson PG. Interleukin-10 gene expression in acute virus-induced asthma. *Am J Respir Crit Care Med* 2005;172:433-439.
- [23] Mallia P, Johnston SL. How viral infections cause exacerbation of airway diseases. *Chest* 2006;130:1203-1210.
- [24] Fleming HE, Little FF, Schnurr D, Avila PC, Wong H, Liu J, Yagi S and Boushey HA. Rhinovirus-16 colds in healthy and in asthmatic subjects: similar changes in upper and lower airways. *Am J Respir Crit Care Med* 1999;160:100-108.
- [25] Vercelli D, Jabara HH, Arai K, Yokota T and Geha RS. Endogenous interleukin 6 plays an obligatory role in interleukin 4-dependent human IgE synthesis. *Eur J Immunol* 1989;19:1419-1424.
- [26] Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST and Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005;201:937-947.
- [27] Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, Kebabdz T, Mallia P, Stanciu LA, Parker HL, Slater L, Lewis-Antes A, Kon OM, Holgate ST, Davies DE, Kolenko SV, Papi A and Johnston SL. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med* 2006;12:1023-1026.
- [28] Schroth MK, Grimm E, Frindt P, Galagan DM, Konno SI, Love R and Gern JE. Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1999;20:1220-1228.
- [29] Kameyoshi Y, Dorschner A, Mallet AI, Christophers E and Schroder JM. Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med* 1992;176:587-592.
- [30] Sanders SP, Proud D, Permutt S, Siekierski ES, Yachechko R and Liu MC. Role of nasal nitric oxide in the resolution of experimental rhinovirus infection. *J Allergy Clin Immunol* 2004;113:697-702.
- [31] Bochner BS and Busse WW. Allergy and asthma. *J Allergy Clin Immunol* 2005;115:953-959.
- [32] Benbernou N, Esnault S, Shin HC, Fekkar H and Guenounou M. Differential regulation of IFN-gamma, IL-10 and inducible nitric oxide synthase in human T cells by cyclic AMP-dependent signal transduction pathway. *Immunology* 1997;91:361-368.
- [33] Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan T, de Waal-Malefyt Rd R, Bazan F, Kastelein RA and Liu YJ. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3:673-680.
- [34] Erickson M, Morkowski S, Lehar S, Gillard G, Beers C, Dooley J, Rubin JS, Rudensky A and Farr AG. Regulation of thymic epithelium by keratinocyte growth factor. *Blood* 2002;100:3269-3278.
- [35] Li M, Hener P, Zhang Z, Kato S, Metzger D and Chambon P. Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopietin in mouse keratinocytes and trigger an atopic dermatitis. *Proc Natl Acad Sci USA* 2006;103:11736-11741.
- [36] Allakhverdi Z, Comeau MR, Jessup HK, Yoon

- BR, Brewer A, Chartier S, Paquette N, Ziegler SF, Sarfati M and Delespesse G. 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.
- [37] Rimoldi M, Chieppa M, Salucci V, Avogadri F, Sonzogni A, Sampietro GM, Nespoli A, Viale G, Allavena P and Rescigno M. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol* 2005;6:507-514.
- [38] Kato A, Favoreto S Jr, Avila PC and Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. *J Immunol* 2007; 179:1080-1087.
- [39] Lee HC and Ziegler SF. Inducible expression of the proallergic cytokine thymic stromal lymphopoietin in airway epithelial cells is controlled by NFkappaB. *Proc Natl Acad Sci USA* 2007;104:914-919.
- [40] Ebner S, Nguyen VA, Forstner M, Wang YH, Wolfram D, Liu YJ and Romani N. Thymic stromal lymphopoietin converts human epidermal Langerhans cells into antigen-presenting cells that induce proallergic T cells. *J Allergy Clin Immunol* 2007;119:982-990.
- [41] Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, Gyarmati D, Aye T, Campbell DJ and Ziegler SF. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;6:1047-1053.
- [42] Al-Shami A, Spolski R, Kelly J, Keane-Myers A and Leonard WJ. A role for TSLP in the development of inflammation in an asthma model. *J Exp Med* 2005;202:829-839.
- [43] Yoo J, Omori M, Gyarmati D, Zhou B, Aye T, Brewer A, Comeau MR, Campbell DJ and Ziegler SF. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal lymphopoietin transgene specifically in the skin. *J Exp Med* 2005;202:541-549.
- [44] Reche PA, Soumelis V, Gorman DM, Clifford SM, Johnston J, Liu YJ, Spits H, de Waal Malefyt R, Kastelein RA and Bazan JF. Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. *J Immunol* 2001;167:336-343.
- [45] Rochman I, Watanabe N, Arima K, Liu YJ, Leonard WJ. Cutting edge: direct action of thymic stromal lymphopoietin on activated human CD4+ T cells. *J Immunol* 2007;178: 6720-6724.
- [46] Isaksen DE, Baumann H, Zhou B, Nivollet S, Farr AG, Levin SD and Ziegler SF. Uncoupling of proliferation and Stat5 activation in thymic stromal lymphopoietin-mediated signal transduction. *J Immunol* 2002;168:3288-3294.