

Short Communication

Increased serum thymic stromal lymphopoietin in children with atopic dermatitis

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The present study investigated the relationship between serum thymic stromal lymphopoietin (TSLP) levels and the presence and severity of atopic dermatitis (AD). Serum TSLP levels, blood eosinophil counts, and serum total and specific immunoglobulin E (IgE) levels were measured in 232 children. Subjects were characterized as having atopic eczema (AE; $n = 75$), non-atopic eczema (NAE; $n = 70$), or normal controls ($n = 87$). Serum TSLP levels in children with AD were significantly higher than normal controls but there were no differences in children with atopic and non-atopic eczema. However, serum TSLP levels in children with AD were not significantly correlated with disease severity, blood eosinophil counts and serum total IgE levels. Our findings show an association between TSLP and AD including both AE and NAE. It is suggested that TSLP may play a contributory role in the pathogenesis of AD regardless of the presence of atopy.

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Atopic dermatitis (AD) is an eczematous, relapsing, highly pruritic chronic inflammatory skin disease. There are at least two forms of AD, atopic eczema (AE, extrinsic) and non-atopic eczema (NAE, intrinsic) (1).

The pathogenesis of AD is not completely understood, although several cell types, such as T lymphocytes, Langerhans cells (LC), eosinophils, and keratinocytes, and factors including cytokines and immunoglobulins, particularly immunoglobulin E (IgE), have been implicated. Recent compelling evidence indicates that thymic stromal lymphopoietin (TSLP) may have a determinant role in the initiation and maintenance of allergic immune response (2). TSLP, an epithelial cell-derived cytokine, can potently activate immature CD11c⁺ myeloid dendritic cells (DC), which subsequently prime CD4⁺ T cells to produce allergy-promoting cytokines such as interleukin (IL)-4, IL-5, IL-13 and TNF- α , but down-regulate IL-10 and interferon (IFN)- γ (3).

The aim of this study was to investigate the relationship between serum TSLP levels and the presence and severity of AD. We also measured serum TSLP levels in subjects with AE and NAE separately and further analyzed the correlation between serum TSLP levels, total eosinophil counts, and serum IgE levels.

Materials and methods

Sera were obtained from 232 subjects (4.0 ± 2.9 yr, 3 months to 14 yr; 124 males and 108 females) who visited the allergy clinic for AD or a general work-up at Severance Hospital of Yonsei University. Seventy-five subjects with AE, 70 with NAE, and 87 normal controls were included in this study. All patients with AD were given a diagnosis according to the revised criteria of Hanifin and Rajka (4) and were treated with topical corticosteroids in combination with or without oral antihistamines. Subjects with AD

who had asthma or allergic rhinitis were excluded because of the possible effect of respiratory allergy to chemokine levels. Disease severity of AD was determined using the SCORing atopic dermatitis (SCORAD) index (5). Pediatric allergists made the AD diagnosis and performed SCORAD. In this study, the patients with AD were divided into those with AE and NAE. Children with AE were sensitive to more than one of six common allergens, and their total IgE levels were more than 100 IU/mL. A specific IgE test was performed with *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, egg whites, cow milk, peanuts, and soybeans, which are six common allergens in Korea. For sera of NAE, total IgE levels were <100 IU/mL, with no detectable specific IgE antibodies to any of the six common allergens. Controls were recruited among subjects for general health work-up. They did not have any history of allergic or inflammatory disease.

This study was approved by the Severance Hospital Institutional Review Board committee. Written consent for participation was obtained from parents with verbal assent from children. At the initiation of the study, total serum IgE levels and peripheral blood eosinophil counts were measured. Total and specific IgE levels were measured using the CAP system FEIA (Pharmacia Diagnostics, Uppsala, Sweden). According to the manufacturer's instructions, specific IgE levels above 0.35 kU/L were considered positive. After venous blood was drawn, serum was separated and stored at -20°C until use. Serum levels of TSLP were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Age, SCORAD index, total IgE level, and total eosinophil counts were expressed as mean $\pm$ s.d and evaluated the differences among the three groups using ANOVA. Serum TSLP levels were expressed as median and interquartile ranges. Differences in them among the groups were evaluated using Kruskal–Wallis test. Correlations between serum TSLP levels and SCORAD index, eosinophil counts, and serum IgE levels were evaluated using Spearman's correlation. Statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). p-values of <0.05 were considered to be statistically significant.

Results

The mean age of subjects with AE was older than those with NAE and normal controls but there

were no significant differences. There were also no significant differences in sex ratio among the groups. SCORAD indices, eosinophil counts, and total IgE levels of subjects with AE were higher than those of subjects with NAE and normal controls in this study (Table 1).

Serum TSLP levels in subjects with AE [27.88 (15.92–50.30) pg/mL] and those with NAE [26.19 (15.54–44.82) pg/mL] were significantly higher than those in normal controls [17.80 (12.70–32.04) pg/mL, $p = 0.002$]. There were no significant differences in serum TSLP levels between children with AE and NAE (Fig. 1).

Serum TSLP levels in AD, including both AE and NAE sub-group, did not show significant correlation with SCORAD index, eosinophil count and total IgE level (data not shown).

Table 1. Demographics of subjects

	AE (n = 75)	NAE (n = 70)	Normal controls (n = 87)
Age (yr)	5.4 $\pm$ 2.9	4.2 $\pm$ 2.8	4.6 $\pm$ 3.1
Sex (M/F)	37/38	35/35	52/35
SCORAD Index	43.6 $\pm$ 33.2*	33.4 $\pm$ 27.7	
Eosinophil count (per μl)	687.6 $\pm$ 798.9*,**	242.1 $\pm$ 149.7	193.9 $\pm$ 125.7
Total IgE (IU/ml)	815.5 $\pm$ 1069.4*,**	28.4 $\pm$ 26.6	32.38 $\pm$ 25.8

Results are indicated as mean $\pm$ s.d.

SCORAD, SCORing atopic dermatitis; NAE, non-atopic eczema; AE, atopic eczema.

Normal reference range of eosinophil count is 0–500/ μl .

* $p < 0.05$ between subjects with AE and NAE.

** $p < 0.05$ between subjects with AE and normal controls.

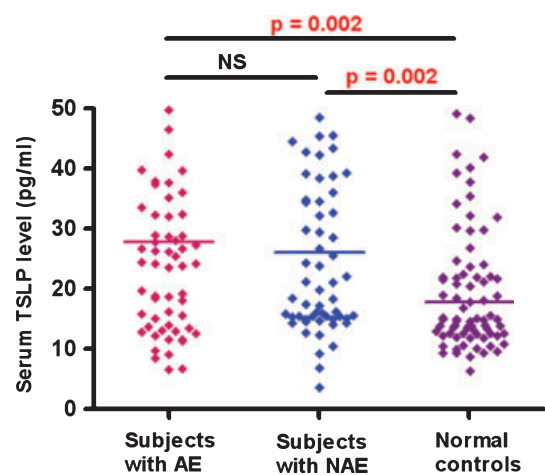


Fig. 1. Comparison of serum thymic stromal lymphopoietin (TSLP) levels in subjects with atopic eczema (AE), non-atopic eczema (NAE) and normal controls. Serum TSLP levels in subjects with AE and NAE were significantly increased compared with normal controls. Bars are shown as medians.

Discussion

Thymic stromal lymphopoietin is epithelial cell-derived cytokine and has emerged as an important factor correlated with AD. High expression of TSLP was observed in keratinocytes of acute and chronic AD (3). Selective ablation of retinoid X receptors (RXR) in epidermal keratinocytes induced rapid TSLP expression and triggered AD in mice (6). Based on this evidence, serum TSLP levels were measured in 145 subjects with AD and 87 normal controls in this study. Serum TSLP levels in subjects with AD were significantly higher than levels in normal controls. This result showed that TSLP might be related to AD *in vivo*. There are some studies about expression of TSLP mRNA or TSLP production measured by using immunohistology on tissue cryosection but to our knowledge, this is the first report to investigate serum TSLP.

However, serum TSLP levels in AD did not show any correlation with disease severity (SCORAD index), eosinophil count, and total IgE level in our study. The results of the present study correspond well with earlier studies, which reported that no correlations were found between the intensity of TSLP staining of an AD lesion and clinical severity or blood IgE level (3). We presume that the serum TSLP is involved only in the initiation of AD and consequently it may not have due impact on the disease severity.

The immunopathogenesis of NAE is different from AE. In AE, cutaneous T cells that produce IL-4, -5 and -13 dominate in the acute phase of the disease, and T cells that produce IFN- γ predominate in the chronic phase of the disease. A prominent feature of AE is cutaneous infiltration with highly reactive cells of dendritic lineage. These include LC and epidermal DC, both of which display the high-affinity receptor for the Fc region of IgE (Fc ϵ RI) on their surface. In NAE, cutaneous T cells produce similar amounts of IL-5 and IFN- γ but less of the Th2 cytokines IL-4 and -13. As with AE, the skin of subjects with NAE harbors a large number of epidermal DCs, which display characteristically lower surface expression of Fc ϵ RI (7). Thus, we investigated subjects with AE and NAE separately. In this study, serum TSLP levels in subjects with AE and NAE were significantly increased compared with the levels of normal controls and there were no significant differences between the two groups with AD despite the humoral and cellular differences.

There is strong evidence suggesting that TSLP plays a role in the pathophysiology of allergic disease. TSLP is produced by epithelial and

stromal cells that are present at the site of allergen entry (3). Mast cells activated by monoclonal antibodies that cross-link high-affinity IgE receptors express very high levels of TSLP (8). DC activated with TSLP induce the generation of CD4⁺ Th cells with a very unique cytokine profile including IL-4, -5, -13 and TNF- α but low or decreased levels of IL-10 and IFN- γ . TSLP was highly expressed by keratinocytes in AD lesions and its expression was associated with the migration and activation of LC, suggesting that TSLP might be an early trigger for DC-mediated allergic inflammation (3).

Several recent studies may explain the elevation of serum TSLP levels in subjects with NAE. A direct functional link among epithelial cells, LC, and T cell-mediated immune responses were reported (9). It was shown that proinflammatory (TNF- α or IL-1 α) and Th2 (IL-4 or -13) cytokines synergized to induce the production of TSLP in human skin explants, but TSLP production could not be inhibited by factors regulating Th2 inflammation such as IL-10, TGF- β , or IFN- γ (10). TSLP, synergistically with IL-1 and TNF, stimulates the production of high levels of Th2 cytokines by human mast cells and is released by primary epithelial cells in response to clinically relevant stimuli such as certain microbial products, physical injury, or inflammatory cytokines (11).

The limitation of this study is that we could not show the correlation of serum TSLP and cutaneous TSLP directly. But there was no report about serum TSLP level in other previous studies. As TSLP is circulating mediators, we assumed that there may be indirect correlation between serum TSLP and cutaneous TSLP and further studies about these are needed.

Conclusively, serum TSLP levels in subjects with AE and those with NAE were significantly higher than normal controls. Our findings show an association between TSLP and AD including both AE and NAE. It is suggested that TSLP may play a contributory role in the pathogenesis of AD regardless of the presence of atopy.

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