Increased serum thymic stromal lymphopoietin in children with atopic dermatitis

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Increased serum thymic stromal lymphopoietin in children with atopic dermatitis

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Written by Eun Byoul Lee.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Increased serum thymic stromal lymphopoietin in children with atopic dermatitis

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Objectives : The present study investigated the relationship between serum thymic stromal lymphopoietin (TSLP) levels and the presence and the severity of atopic dermatitis(AD), in subjects with atopic eczema (AE) and nonatopic eczema (NAE) separately.

Patients and Methods : Serum TSLP levels, total serum IgE levels, total eosinophil counts and specific IgE levels were measured in 232 children. The subjects were characterized as having AD (n=75), NAE (n=70), or normal controls (n=87).

Results : Serum TSLP levels in both subjects with AE (27.88 [15.92-50.30] pg/mL) and 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=.002). There were no significant differences in serum TSLP levels between subjects with AE and NAE. Serum TSLP

levels in subjects with AD did not show significant correlation with disease severity (SCORAD index), eosinophil counts and total IgE levels.

Conclusion : Serum TSLP is related to both AE and NAE. Serum TSLP level might be a supportive marker for diagnosis in children with AE and NAE.

Key words : thymic stromal lymphopoietin, atopic dermatitis

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I. INTRODUCTION

Atopic dermatitis (AD) is an eczematous, relapsing, highly pruritic chronic inflammatory skin disease.[1, 2] The prevalence of AD is high, especially in children, and it has been rising in recent decades.[1] There are at least two forms of AD, i.e. an atopic eczema (AE, extrinsic form) and nonatopic eczema (NAE, intrinsic form) of disease.[3] The IgE-mediated variant (AE) is the most prevalent form, related to about 70~85% of the AD patients,[3] and the non-IgE-mediated variant (NAE) has a frequency of approximately 15-40% depending on the location of the study and the parameters used in defining the disorder.[4]

The pathogenesis of AD is not completely understood, although several cell types (eg, T lymphocytes, Langerhans cells, eosinophils, keratinocytes) and factors (eg, cytokines and immunoglobulins, particularly IgE) have been implicated.[5] Recent scientific studies suggest an amplification cycle of atopic skin inflammation (Figure 1).[6] This cycle might start with pruritus, and scratch and mechanical injury induce proinflammatory cytokine and chemokine production.[6] Compelling evidence was recently provided that thymic stromal lymphopoietin

(TSLP) may have a determinant role in the initiation and maintenance of allergic immune response.[7]

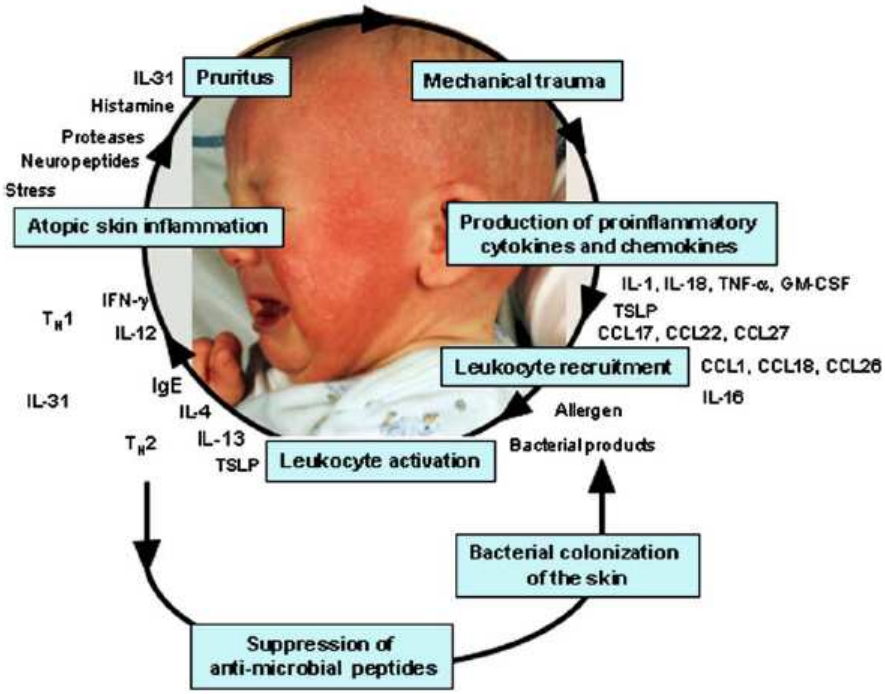

Figure 1. An amplification cycle of atopic skin inflammation. Pruritus represents a prominent symptom of AD. Patients scratch and induce mechanical injury, resulting in proinflammatory cytokine and chemokine. Memory T cell encounter their specific antigen. Epithelial cell-derived cytokines (eg.TSLP) instruct dendritic cells to induce Th2 cell differentiation. Adopted from *Homey B et al. Cytokines and chemokines orchestrate atopic skin inflammation. J Allergy Clin Immunol 2006; 118:178-89.*

TSLP is an IL-7-like cytokine, originally cloned from a murine thymic stromal cell line, that supports the growth and differentiation of B cells and the proliferation of T cells.[8, 9] However, human TSLP, unlikely mouse TSLP, did not support the development or activation of B and T

cells, instead potently activated immature CD11c+ myeloid dendritic cells (DCs). TSLP-activated DCs induce proliferation of naïve CD4+ T cells, which subsequently differentiated into Th2 cells that produced the allergy-promoting cytokines interleukin (IL)-4,IL-5, IL-13 and TNF, but did not produce IL-10 or interferon (IFN)-γ.[10]

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

II. MATERIAL AND METHODS

1.Subjects

Sera were obtained from 232 subjects (4.0±2.9 years, 3 months to 14 years; 124 males and 108 females) who visited the allergy clinic for AD or a general health work-up at the severance Hospital of Yonsei University from January 2002 to May 2004. Seventy-five subjects with AE, 70 with NAE, and 87 normal controls were included in this study. All subjects with AD were diagnosed according to the criteria of Hanifin and Rajka.[11] Subjects with AD who had asthma or allergic rhinitis were excluded because of the possible effect of respiratory allergy to chemokine levels. All subjects were drug-free during the time of the study. Disease severity of AD was determined using the SCORing Atopic Dermatitis (SCORAD) index system.[12, 13]

In this study, subjects with AD were devided into those with AE and NAE.[14] Subjects with AE showed the specific IgE to more than one of six common allergens, *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, egg whites, cow milk, peanuts, and soybeans, or their total IgE levels were more than 100 IU/mL. NAE was defined as subjects with eczema, total IgE levels < 100 IU/mL, and no detectable specific IgE antibodies to any of 6 common allergens for AD.

2. Measurement of total eosinophil count, total and specific IgE levels in serum

At the initiation of 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_A/L were considered positive. After venous blood was drawn, serum was separated and stored at -20℃ until use.

3. Evaluation of serum thymic stromal lymphopoietin

Serum levels of TSLP were measured using commercially available ELISA kits (R&D systems, Mineapolis, MN, USA), according to the manufacturer's instructions.

4. Statistical Analysis

Differences in age, SCORAD index, total IgE level, total eosinophil counts among three groups were evaluated using the ANOVA. Differences in serum TSLP levels among groups were evaluated using the Kruskal-Wallis test. Correlation between serum TSLP levels and SCORAD index, eosionophil counts, serum IgE levels were evaluated using Pearson's correlation. Statistical analyses were done using SPSS version 13.0 (SPSS Inc, Chicago, IL). *P*-values of less than 0.05 were considered to be statistically significant.

III. RESULTS

1. Demographics of subjects

The mean age of subjects with AE was older than those of subjects with NAE and normal controls, but there were no significant differences. There were no significant differences in sex ratio among groups (Table 1). SCORAD indexes, 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. **Demographic of subjects**

	Subjects with AE (n=75)	Subjects with NAE (n=70)	Normal Controls (n=87)
Age (years)	5.4±2.9	4.2±2.8	4.6±3.1
Sex (M/F)	37/38	35/35	52/35
SCORAD index	43.6±33.2*	33.4±27.7	
Eosinophil count (μL^{-1})	687.6±798.9*†	242.1±149.7	193.9±125.7
Total IgE (IU/mL)	815.5±1069.4*†	28.4±26.6	32.38±25.8

Results are indicated as mean ±SD.

* $P < .05$ between subjects with AE and subjects with NAE

† $P < .05$ between subjects with AE and normal controls

SCORAD, SCORing Atopic Dermatitis.

2. The characteristics of sensitive allergens in subjects with atopic eczema

The most commom sensitive allergens in subjects with AE subjects (n=75) were *Dermatophagoides pteronyssinus* (n=51, 68.0%), Dermatophagoides farinae (n=37, 49.3%), egg whites (n=36, 48.0%), cow milk (n=30, 40.0%), peanuts (n=8, 10.7%) and soybean (n=7, 9.3%), in order of frequency (Table 2).

Table 2. The chracteristics of sensitive allergens in subjects with atopic eczema

Allergen	Number of subjects (Total = 75)	%
Dermatophagoides pteronyssinus	51	68.0
Dermatophagoides farinae	37	49.3
Egg whites	36	48.0
Cow milk	30	40.0
Peanuts	8	10.7
Soybean	7	9.3

3. Serum thymic stromal lymphopoietin levels in subjects with atopic eczema, non-atopic eczema and normal controls

Serum TSLP levels in subjects with AE (27.88 [15.92-50.30] pg/mL) and those in subjects 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*= .002). There were no significant differences in serum TSLP levels between children with AE and NAE (Figure 2).

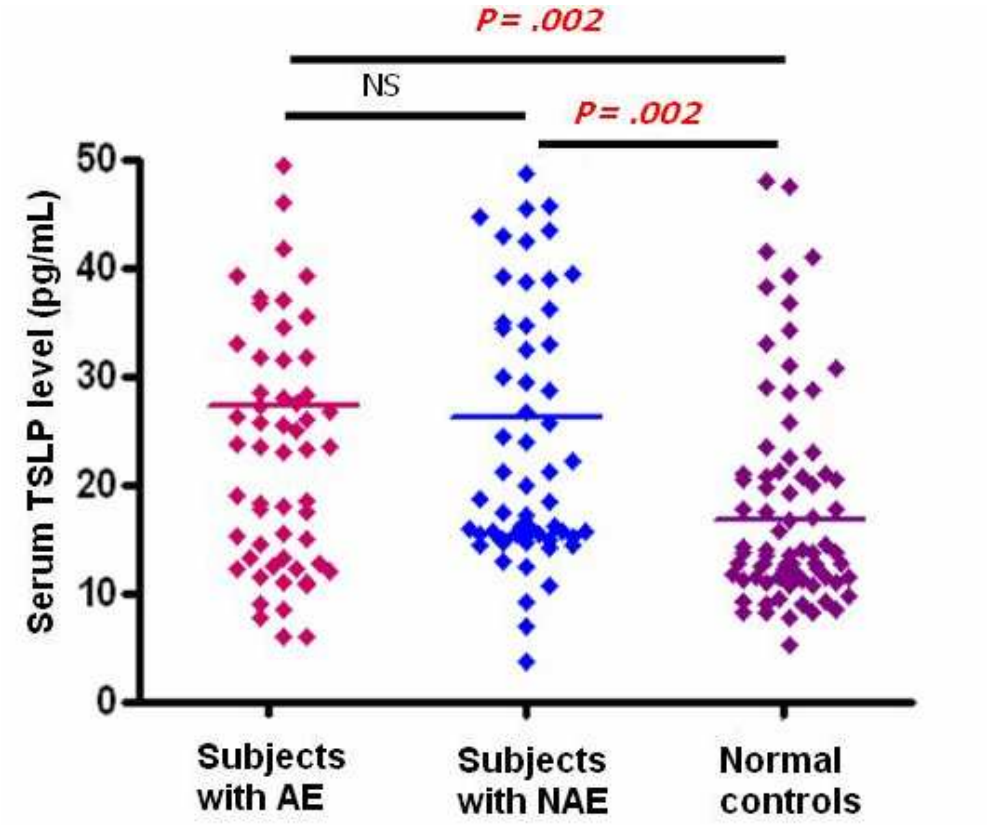

Figure 2. Comparison of serum thymic stromal lymphopoietin (TSLP) levels in subjects with atopic eczema (AE), those with non-atopic eczema (NAE) and normal controls. Serum TSLP levels in subjects with AE and those in subjects NAE were significantly increased compared with those in normal controls. Serum levels of TSLP in subjects with AE, those in subjects with NAE and those in normal controls were 27.88 (15.92-50.30) pg/mL, 26.19 (15.54-44.82) pg/mL and 17.9 (12.70-32.04) pg/mL, respectively. Data are shown as medians (Interquartile range).

4. Correlation of serum thymic stromal lymphopoietin levels with disease severity, eosinophil counts and total immunoglobulin E levels

Serum TSLP levels in AD did not show significant correlation with disease severity (SCORAD index), eosinophil counts and total IgE levels (Table 3).

Table 3. Correlation coefficients between serum thymic stromal lymphopoietin levels and disease severity, eosinophil counts, serum immunoglobulin E levels in subjects with atopic dermatitis

	Correlation coefficient with serum TSLP level(pg/mL)
SCORAD index	- .060
Eosinophil count (μL^{-1})	- .042
Total IgE (IU/mL)	.059

* $P < .05$

IV. DISCUSSION

TSLP is epithelial cell-derived cytokine and has emerged as an important factor correlated with AD. Soulmelis et al.[10] reported that high expression of TSLP was observed in keratinocytes of acute and chronic AD. Li et al.[15] reported that selective ablation of retinoid X receptors (RXRs) in epidermal keratinocytes induced rapid TSLP expression and triggered AD in mice. Based on these evidences, 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 the levels in normal controls. This result showed that TSLP might be related to AD in vivo. There are some studies about the expression of TSLP mRNA or TSLP production measured by using immunohistology on tissue cryosection, but the investigations of serum TSLP are rare.

The immunopathogenesis of NAE is different from that of AE. Histologic differences have not been observed,[4] but there are humoral and cellular differences between the AE and the NAE.[3] In the AE, cutaneous T cells that produce IL-4, IL-5 and IL-13 dominate in the acute phase of disease, and T cells that produce IFN-γ predominate in the chronic phase of the disease.[16] A prominent feature of AE is the cutaneous infiltration with highly reactive cells of dendritic lineage. These cells include Langerhans cells (LCs) and epidermal DCs both of which display the high-affinity receptor for the Fc region of IgE (FcεRI) on their surface. In the NAE, cutaneous T cells produce similar amounts of IL-5 and IFN-γ, but produce less of the Th2 cytokines, IL-4 and IL-13.[17] As with AE, the skin of subjects with NAE harbors a large number of epidermal DCs, which display characteristically lower surface expression of FcεRI.[18] Thus, we investigated subjects with AE and NAE separately.

In this study, serum TSLP levels in subjects with AE and those in subjects with NAE were significantly increased compared with the levels of normal controls, and there were no

significant differences between two groups in subjects with AD despite of the humoral and cellular differences.

There are strong evidences suggesting that TSLP plays a role in the pathophysiology of allergic disease. TSLP is produced by epithelial cells and stromal cells that are present at the entry site of allergen.[10] Mast cells activated by monoclonal antibodies that cross-link high-affinity IgE receptors express very high levels of TSLP.[19] DCs activated with TSLP induce the generation of CD4+ Th cells with a very unique cytokine profile, including IL-4,IL-13,IL-5 and TNF-α, but low or decreased levels of IL-10 and IFN-γ.[10] TSLP was highly expressed by keratinocytes in AD lesions and its expression was associated with the migration and activation of LCs, suggesting that TSLP might be an early trigger for DC-mediated allergic inflammation. [10]

Recent several studies may explain the elevation of serum TSLP levels in subjects with NAE. Ebner et al.[20] showed a direct functional link among epithelial cells, LCs, and T-cell mediated immune responses and acts upstream of CD4+ T cell responses. Bogiatzi et al.[21] showed that proinflammatory (TNF-α or IL-1α) and Th2 (IL-4 or IL-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. Allakhverdi et al.[22] showed that TSLP, synergistically with IL-1 and TNF, stimulated the production of high levels of Th2 cytokines by human mast cells and reported that TSLP is released by primary epithelial cells in response to clinically relevant stimuli, such as certain microbial products, physical injury, or inflammatory cytokines.

These results supported the view that TSLP might be involved in the pathogenesis of AD, whether atopic or not. Thus serum TSLP level might be a supportive marker for diagnosis in both AE and NAE.

In this study, serum TSLP levels in AD did not show any correlation with disease severity

(SCORAD index), eosinophil count and total IgE levels. The results of the present study correspond well with the earlier studies which reported that no correlation were found between the intensity of the TSLP staining on AD lesion and the clinical severity or blood IgE levels.[10]

V. CONCLUSION

Serum TSLP levels in subjects with AE and those in subjects with NAE were significantly higher than those in normal controls. Serum TSLP levels in AD did not show statistically significant correlation with disease severity (SCORAD index), eosinophil counts and total IgE levels. Conclusively, TSLP is related to both AE and NAE, and serum TSLP level might be a supportive marker for diagnosis in subjects with AE and NAE.

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Abstract (in Korean)

아토피피부염 환아에서 혈청 Thymic Stromal Lymphopoietin (TSLP)의 증가

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목적 : Thymic stromal lymphopoietin (TSLP)은 수지세포의 성숙과 활성화를 유도하는 싸이토카인으로, 최근 아토피피부염과의 연관성이 보고되고 있다. 본 연구에서는 혈청 TSLP 의 농도가 소아의 아토피피부염을 진단하고 중증도를 평가하는 지표로 유용한지 여부와 그 의의를 알아보고자 하였다.

대상 및 방법 : IgE 매개성 아토피피부염 환아 75 명, 비 IgE 매개성 아토피피부염 환아가 70 명, 정상 대조군 87 명을 대상으로 하였다. 전체 대상아에서 혈청 TSLP 농도, 총 IgE 농도, 호산구수를 측정하였으며, SCORAD 지수로 중증도를 평가하였다.

결과 : IgE 매개성 아토피피부염 환아에서 총 IgE 농도, 호산구수, SCORAD 지수는 비 IgE 매개성 아토피피부염 환아 및 정상 대조군에 비해 유의하게 높았다($P < 0.05$). TSLP 농도는 IgE 매개성 아토피피부염 환아에서 27.88 (15.92-50.30) pg/mL, 비 IgE 매개성 아토피피부염 환아에서는 26.19 (15.54-44.82) pg/mL, 정상 대조군에서는 17.90 (12.70-32.04) pg/mL 로, IgE 매개성 아토피피부염 환아와 비 IgE 매개성 아토피피부염 환아 두 군간의 통계적인 차이는 없었으나, 두 군 모두 정상 대조군에 비해 유의하게 높았다($P = 0.002$). 그러나 혈청 TSLP 농도는 질병의 중증도, 총 IgE 농도 및 호산구수 사이에 상관관계를 나타내지 않았다.

결론 : 혈청 TSLP 농도는 소아의 IgE 매개성 아토피피부염과 비 IgE 매개성 아토피피부염 모두에서 진단에 도움이 되는 지표로 사용될 수 있을 것으로 생각된다.

핵심되는 말 : thymic stromal lymphopoietin (TSLP), 아토피피부염