Published in final edited form as:

Ann Allergy Asthma Immunol. 2010 November ; 105(5): 403–404. doi:10.1016/j.anai.2010.09.017.

Increased affected skin gene expression and serum levels of thymic stromal lymphopoietin in atopic dermatitis

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Atopic dermatitis (AD) is a chronic highly pruritic inflammatory skin disease, which has increased considerably in industrialized countries.¹ AD is characterized by allergic inflammation in the skin,² and has a strong association with other atopic disorders such as asthma and allergic rhinitis.¹

Thymic stromal lymphopoietin (TSLP) is a cytokine closely related to IL-7,³ mostly produced by epithelial cells and keratinocytes in response to infection, trauma and inflammation. TSLP mediates Th2 responses through activation of dendritic cells, and has emerged as an important regulator of allergic inflammation. TSLP also activates mast cells to release several cytokines, such as IL-5, IL-6 and IL-13, but only in the presence of IL-1

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Authors' contributions: KDA and TCT conceived and designed the study, generated, analyzed and interpreted the data, and prepared the manuscript. AA, MV and BZ helped generate and analyze the data, and revise the manuscript. DK and AKK participated in study design, analysis and interpretation of the data, and revised the manuscript.

Conflicts of interest: The authors declare no conflicts of interest.

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and TNF.⁴ One paper reported increased TSLP protein expression in lesional skin of AD patients.³

We investigated gene expression of TSLP in lesional skin biopsies and serum levels of AD patients (n=9, mean age $\pm$ 1 SD=41.33 $\pm$ 21.40; three males, six females) compared to controls (n=9, mean age $\pm$ 1 SD=42.8 $\pm$ 18.1; three males, six females).

All 10 mm punch skin biopsies were obtained (abdomen, arm, back, hand, and neck; there was no apparent correlation between any of these sites and TSLP levels) from patients who had mild, localized, chronic AD, and from controls (back). No subject had received any anti-inflammatory medication for 15 days prior to the biopsy. Contact dermatitis, asteatotic eczema or other types of dermatitis were excluded. The Medical Ethics Committee of University of Athens Medical School approved this protocol. All participants gave their written informed consent according to the Declaration of Helsinki Principles. Patients were free of other major medical conditions except allergic rhinitis as noted. All biopsies were placed in RNeasy lysis buffer (Qiagen, Crawley, UK) and stored at -20°C . Total RNA from skin biopsies was extracted by TRIzol isolation method (Invitrogen, Carlsbad, CA). cDNA was synthesized using iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA). Quantitative PCR was performed using Taqman probes (Applied Biosystems, Foster City, CA) on a 7300 Real Time PCR System (Applied Biosystems). For qPCR, the cDNA was reverse-transcribed from 300 ng RNA from each sample. Relative mRNA levels were normalized to the GAPDH endogenous control. Data were analyzed with the non-parametric Mann-Whitney *U* test, since values did not conform to a normal distribution. Serum measurements are reported in pg/ml.

Relative TSLP gene expression was significantly increased in the lesional AD skin samples (median=4.93; range=2.27–23.99) as compared to controls (median=0.84; range=0.49–1.85) (Fig. 1a, $p<0.0001$).

Blood was obtained the same day prior to the biopsy and the serum was isolated, aliquoted and stored at -20°C until use. TSLP levels were determined with ELISA (R&D Systems, Minneapolis, MN). There was a statistically significant increase in TSLP levels in patients with AD (median=130.5; range=0–2,246), in contrast to the controls (median=0; range=0–62.5) (Fig. 1b, $p=0.0056$). The 3 female patients with the highest serum levels (2246.6, 859.4, and 768.2 pg/ml) also had allergic rhinitis confirmed by skin prick testing.

There was neither a positive nor a negative correlation between skin TSLP gene expression and serum levels in any of the subjects. Local skin TSLP gene expression and production may be one of the earliest steps in the inflammatory process, preceding systemic increases in TSLP levels. Our results contrast with another human study reporting that TSLP serum levels were not increased in AD (n=46; average age=26.5 years),⁵ but are in agreement with increased TSLP serum levels in children with atopic eczema (n=75) and non-atopic eczema (n=70), as compared to normal controls (n=87).⁶ All of our patients had mild, localized chronic AD and, therefore, do not permit an attempt to correlate with severity of disease. Besides, this study was not powered for such analysis.

The highest serum values corresponded to AD patients with concomitant allergic rhinitis confirmed with skin prick testing. This finding supports the hypothesis that increase of serum TSLP levels may also precede other atopic diseases, such as allergic rhinitis and asthma. In fact, a study showed that skin-derived TSLP contributed to the aggravation of asthma in mice.⁷ The role of TSLP in human asthma is supported by another study, which showed that bronchial TSLP expression was increased in asthmatics (n=20) compared to controls (n=15) and correlated with disease severity.⁸ Based on the apparent association between increased serum TSLP levels and allergic rhinitis, investigation of atopic subjects

without AD could provide further insight into the relationship between TSLP and allergic disease.

Acknowledgments

Funding sources: Aspects of this work were funded by NIH grant AR47652 to TCT.

References

1. Bieber T. Atopic dermatitis. *N Engl J Med* 2008;358:1483–1494. [PubMed: 18385500]
2. Metz M, Maurer M. Innate immunity and allergy in the skin. *Current Opinions in Immunology* 2009;21:687–693.
3. Soumelis V, Reche PA, Kanzler H, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3:673–680. [PubMed: 12055625]
4. Allakhverdi Z, Comeau MR, Jessup HK, et al. Thymic stromal lymphopoietin is released by human epithelial cell in response to microbes, trauma, or inflammation and potently activates mast cells. *J Exp Med* 2007;19:253–258. [PubMed: 17242164]
5. Nakamura K, Tsuchida T, Tsunemi Y, Saeki H, Tamaki K. Serum thymic stromal lymphopoietin levels are not elevated in patients with atopic dermatitis. *J Dermatol* 2008;35:546–547. [PubMed: 18789077]
6. Lee EB, Kim KW, Hong JY, Jee HM, Sohn MH, Kim KE. Increased serum thymic stromal lymphopoietin in children with atopic dermatitis. *Pediatr Allergy Immunol* 2010;21:e457–e460. [PubMed: 20444170]
7. Zhang Z, Hener P, Frossard N, et al. Thymic stromal lymphopoietin overproduced by keratinocytes in mouse skin aggravates experimental asthma. *Proc Natl Acad Sci U S A* 2009;106:1536–1541. [PubMed: 19188585]
8. Ying S, O'Connor B, Ratoff J, 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. [PubMed: 15944327]

Figure 1.

Scattergrams of (a) TSLP gene expression determined in skin by qPCR and (b) TSLP levels in serum measured by ELISA from controls and atopic dermatitis patients. The value for TSLP gene expression from one patient was not included because the quality of mRNA was poor, as also evidenced by low GAPDH expression; inclusion of that value would have yielded even higher results for the patient group.