Effects of Thymic Stromal Lymphopoietin on Invariant Natural Killer T Cell in Atopic Dermatitis

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Effects of Thymic Stromal Lymphopoietin on Invariant Natural Killer T Cell in Atopic Dermatitis

Directed by Professor Kwang Hoon Lee

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ABSTRACT

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(Directed by Professor Kwang Hoon Lee)

Thymic stromal lymphopoietin (TSLP) is one of the most important cytokines regarded as trigger of atopic dermatitis (AD). TSLP expression is highly increased in the epidermis of AD patients. The increased expression of TSLP is specific in AD. Although many studies showed the effect of TSLP on dendritic cells (DC) activation, no reports have shown the effect on human invariant natural killer T (iNKT) cells. iNKT cells are one of the potent immune modulators acting through massive production of various cytokines including interlukin-4 (IL-4) and interferon-γ (IFN-γ).

The aim of this study is to investigate the difference between the population of iNKT cells in peripheral blood mononuclear cells (PBMCs) of normal

controls (NC) and of AD patients, to confirm the expression of TSLP in the lesional skin of AD patients and clarify the effect of TSLP on iNKT cells and the association of activated iNKT cells in the pathogenesis of AD.

In the flow cytometic analysis, the population of iNKT cells in PBMCs was highly increased in AD patients. However, there's no correlation between iNKT cell population and severity of AD, also between iNKT cell population and IgE level in AD patients. And the population of iNKT cells in PBMCs was not affected by TSLP-treated DC. The cultured keratinocytes expressed TSLP, however IL-4, tumor necrosis factor α (TNFα), transforming growth factor-β (TGF-β), keratinocyte growth factor (KGF) did not change TSLP expression on both western blot and RT-PCR. In the immunohistochemistry, high expression of TSLP was observed in the epidermis of the lesional skin of AD patients. iNKT cells expressed high level of TSLP receptor and TSLP-treated PBMCs influenced on the IL-4 secreting iNKT cells. The expression of IL-4 was increased when they were treated with TSLP. TSLP not only activated DC but also activated iNKT cells to express TH2 cytokine such as IL-4. On the contrast, IFN-γ, TH1 cytokine did not show significant changes by the treatment of TSLP. These results correspond to the findings that AD patients have an increased number of cells expressing IL-4 and IL-13, but not IFN-γ.

In conclusion, expression of TSLP in AD might activate iNKT cells to secrete TH2 cytokine IL-4 and thus activated iNKT cells might be involved in TH2 immune responses of AD.

Key words: atopic dermatitis, thymic stromal lymphopoietin, invariant natural killer T cell

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I. INTRODUCTION

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease[1,2]. Complex interactions among genetic, environmental, skin barrier, pharmacologic, and immunologic factors contribute to the pathogenesis of AD[3-5]. The key role of immune effector T cells in AD is supported by the observation that primary T-cell immunodeficiency disorders frequently show increased serum IgE levels and eczematous skin lesions, which clear after successful bone marrow transplantation[6-10]. The important role of TH1 and TH2 cytokines in the skin inflammatory response has been demonstrated in experimental models of allergen-induced skin inflammation using targeted deletions or over-expression of these cytokines[11,12]. In this regard, transgenic

mice genetically engineered to over-express interleukin-4 (IL-4) in their skin have inflammatory pruritic skin lesions similar to those seen in patients with AD, suggesting that local skin expression of TH2 cytokines plays a critical role in AD[1,13].

TH1 and TH2 cytokines contribute to the development of skin inflammation in AD[14-17]. As compared with the skin of normal controls, even unaffected skin of AD patients has an increased number of cells expressing IL-4 and interleukin 13 (IL-13), but not interferon- γ (IFN-γ). In addition, IFN-γ and IL-4 themselves are involved in the differentiation of TH1 and TH2 cells, respectively, and these two subsets of helper T cells cross-regulate each other[18]. Fine-tuning of the TH1/TH2 balance is important to avoid immune dysfunction and AD relapse.

Invariant natural killer T cells (iNKT cells) constitute an unique and evolutionally conserved subpopulation of T lymphocytes that express both T cell receptor (TCR) and NK receptors such as Vα14-Jα18 segments in mice and Vα24-Jα18 segments in humans[19-21]. iNKT cells recognize glycolipid antigens presented by nonpolymorphic major histocompatibility complex (MHC) class I-like protein, CD1d[22]. Human Vα24+ iNKT cells have been shown to produce large amounts of IFN-γ and IL-4[23] that lead to downstream activation of dendritic cells (DC), NK cells, B cells, and conventional T cells.

Through this property, iNKT cells have been shown to promote the polarization of conventional $CD4^+$ T cells into TH1 and TH2 cells and to regulate different types of immune responses[24-27]. Several *in vivo* models demonstrated that, upon intentional or natural activation, iNKT cells are extremely flexible in nature and can either suppress or enhance immune-mediated diseases, including inflammation, cancer, and autoimmune diseases. Thus, $V\alpha24^+$ iNKT cells potentially influence the determination of the TH1/TH2 profile and therefore could affect the atopic state[27-29].

Human thymic stromal lymphopoietin (TSLP) is a novel IL-7-like cytokine produced by human epithelial, stromal and mast cells[30,31]. The TSLP receptor is a heterodimer that consists of the IL-7 receptor α chain (IL-7R) and a common γ-like receptor chain called TSLP receptor (TSLPR)[32-35]. TSLPR is a member of the hematopoietin receptor family and is required for signaling by TSLP[33]. Human TSLP potently activated human CD11c+ DC, which subsequently primed naive T helper cells to produce high concentrations of IL-13, IL-5 and TNFα and moderate amounts of IL-4 and down-regulate IL-10 and IFN-γ[31]. Human TSLP activates DC, but does not appear to have any direct biological effects on B cells, T cells, neutrophils or mast cells. This is accordance with the expression of TSLPR mRNA in $CD11c^+$ DC, but not in other cell types. It has been reported that TSLP is highly expressed in the

lesional keratinocytes of AD but not in the non-lesional keratinocytes of AD or other diseases involving skin inflammation. Thus, TSLP represents a key epithelial cell or keratinocyte-derived cytokine that directly triggers DC-mediated allergic inflammation[36-38]. Though many studies have been performed to clarify the effect of TSLP on DCs, it is not clear whether TSLP can activate iNKT cells and affect in the pathogenesis of AD.

The aim of this study is to investigate the difference between the population of iNKT cells in peripheral blood mononuclear cells (PBMCs) of normal controls (NC) and to AD patients, to confirm the expression of TSLP in the lesional skin of AD patients and clarify the effect of TSLP on iNKT cells and the association of activated iNKT cells in the pathogenesis of AD.

II. Materials and Methods

1. Patients

Blood samples and skin tissues were obtained with informed consent from sixteen AD patients according to the criteria of Hanifin and Rajka[39]. Thirteen non-atopic healthy donors and three psoriasis patients were also included as NC and patient control. The AD patients have not received any systemic treatment except for topical steroid for at least four weeks before collection of blood sample. Severity of AD patients was evaluated by Eczema Area and Severity Index (EASI) score[40]. The institutional review board approved this study.

2. Sorting of human iNKT cell

1) Immunofluorescence and cell sorting: Isolated PBMCs were stained with 10 μg/ml mouse anti-human Vα24 monoclonal antibody (Abcam plc., Cambridge, UK) for 30 minutes. FITC-conjugated anti-mouse IgG secondary antibody was added after washing and cells were incubated for 30 minutes at 4 ^{o}C. Cells were sorted by epic altra cell sorter (Beckman Coulter Inc., Fullerton, CA, USA) and then sorted cells were cultured in 10% RPMI 1640 (Gibco laboratories, Grand Island, NY, USA).

2) Magnetic cell sorting (MACS): Total human PBMCs were stained with 10 μg/ml mouse anti-human Vα24 monoclonal antibody (Abcam) for 30 minutes. After washing, cells were passed through LS column. Cell suspension was centrifuged at 300xg for 10 minutes. Cells were resuspended in 80 μl of EDTA/BSA/PBS MACS buffer. Anti-mouse IgG beads were added to 10^7 cells and cells were incubated for 15 minutes at 4 ^{o}C. LS column was placed in the magnetic field of a suitable MACS separator and rinsed once with 3 ml buffer. Cells were resuspended up to 500 μl of buffer and applied onto the column. Column was washed with 3 ml buffer for 3 times and the cells which passed through were removed. Column was separated from magnetic field and 5 ml buffer was pipetted onto the column. The flushed out fraction with the magnetically labeled cells were collected and cultured in 10% RPMI 1640 (Gibco).

3. Analysis of iNKT cell population in NC and AD patients

1) Flow cytometric analysis: Whole blood was obtained from NC and AD patients. PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation. The isolated PBMCs were stained by mouse anti-human Vα24 antibody (Abcam) for 1 hour at 4^{o}C and incubated with anti-mouse IgG secondary antibody (Abcam) for 1 hour at 4^{o}C. The stained PBMCs were analyzed by FACSCalibur (Becton Dickinson Mountain View, CA USA). the

percentage of Vα24-positive cells in 1 x 10^4 cells were regarded as the population of iNKT cells in PBMCs

2) Culture of human DC: The culture media contain RPMI 1640 (Gibco), 2 μM L-glutamine (Gibo), 100 IU/ml penicillin (Gibo), 100 μg/ml streptomycin (Gibo), and 10% fetal bovine serum (Hyclone, Logan, UT, USA). Monocytes from study participants were isolated from PBMCs via the cell attachment method. PBMCs were attached to six-well plates for 40 minutes. The supernatant and the floating cells were discarded, and the attached cells were used for culture. Monocytes were plated in six-well plates at a final concentration of $3x10^6$ cells in 3 ml of culture medium. DC were generated by culturing monocytes for six days in medium supplemented with 500 U/ml GM-CSF and 1000 U/ml IL-4 at days 0, 2, 4, 6. One third of the medium was removed, and an equivalent volume of fresh medium was added with the cytokines.

3) iNKT cell population in PBMCs co-cultured with TSLP-treated and TSLP-untreated DC: For activation of DC, cells were treated with 10 ng/ml TSLP (R&D Systems. Minneapolis, MN, USA) for 24 hours at the last day of culture. TSLP-treated and TSLP-untreated DCs were co-cultured with PBMCs for 24 hours. The population of Vα24-positive cell was analyzed by flow cytometry as mentioned above.

4. Analysis of TSLP expression in the keratinocytes and on the skin of NC and lesional skin of AD patients

1) Culture of keratinocytes and cytokine treatment: The primary cultured human keratinocytes were purchased from Cambrex (Cambrex, Walkersville, MA, USA). Cells were cultured with KGM-2 (Cambrex) at 37^{o}C in CO_2 incubator. The keratinocytes were divided into six well plate and 800 U/ml IL-4, 25 ng/ml KGF, 10 ng/ml TGF-β, 20 ng/ml TNFα was added into each well and cultured for 16 hours.

2) RT-PCR analysis for TSLP mRNA expression in cultured keratinocytes: To identify whether TSLP expressed in keratinocytes treated with several kinds of cytokine. Total RNA was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). cDNA synthesis and gene amplifications were performed as described by the PCR kit protocol (Qiagen, Valencia, CA, USA). The mixture was incubated at 95^{o}C for 15 minutes before the first cycle and extended at 72^{o}C for 10 minutes after all cycles were completed. PCR products were visualized on 2% agarose gels containing 5 μg/ml ethidiumbromide.

3) Western blot analysis for TSLP expression of cultured keratinocytes: To investigate the expression of TSLP in cultured keratinocytes, western blot analysis was performed. The cultured keratinocytes were homogenized with a

pro-prep lysis buffer (Intron, Seoul, Korea), and the concentration of cellular protein was determined using a Bio-Rad assay reagent (Bio-Rad, Hercules, CA, USA). Equal concentrations of cellular protein mixed with a 5 x sample buffer were heated at 100^{o}C for 5 minutes and separated on 10% SDS-polyacrylamide gels. After electrophoresis, the proteins were transferred onto PVDF membranes with Tris buffer (0.025 M Tris-HCI, 0.192 M glycine, and 20% MeOH). The membrane was blocked for 1 hour at room temperature with 5% skim milk in TBS-Tween 20. Membranes were incubated overnight at 4^{o}C with specific antibodies, and then incubated with horseradish peroxidase-conjugated anti-mouse antibody for 1 hour at room temperature. The membranes were then incubated with an enhanced chemiluminescence system detection kit (Amersham Life Science, Arlington Height, IL, USA).

4) Immunohistochemical staining for TSLP expression in the lesional skin of AD patients: To demonstrate the expression of TSLP in the lesional skin of AD patients, immunohistochemical staining was performed. The frozen sectioned skin of NCs, psoriasis and AD patients were fixed in acetone for 10 minutes. The sections were incubated for 10 minutes in 10% normal goat serum to block non-specific binding. After blocking the sections were incubated in mouse monoclonal TSLP antibody (Abcam) for 1 hour at room temperature. Biotinylated anti-mouse antibody (Zymed co, Carlsbad, CA,

USA) for TSLP was then used to detect the primary antibodies, and the sections were incubated in peroxidase-conjugated streptavidin (Zymed) for 30 minutes at room temperature. Peroxidase activation was visualized with DAB solution.

5. Immunohistochemical staining for the expression of TSLP and Vα24 in the lesional skin of AD patients

In order to determine if the TSLP and Vα24 were expressed concurrently in the same skin of AD patients, double staining was performed in NC and AD skin. The sections were fixed in acetone for 10 minutes. The fixed sections were incubated in mouse monoclonal Vα24 antibody (Abcam) at room temperature for 30 minutes. Biotinylated anti-mouse antibody (Zymed) were then used to detect the primary antibodies, and the sections were incubated in alkaline phosphatase-conjugate (Zymed) for 30 minutes at room temperature. Alkaline phosphatase activation was visualized with substrate-chromogen mixture solution. The sections were incubated in double staining enhancer reagent (Zymed) at room temperature for 30 minutes and then were incubated in mouse monoclonal TSLP antibody (R&D Systems) at room temperature for 30 minutes. Biotinylated anti-mouse antibody for TSLP were then used to detect the primary antibodies, and the sections were incubated in peroxidase-conjugated streptavidin for 30 minutes at room temperature. Peroxidase

activation was visualized with DAB solution. As negative controls, the primary antibodies were substituted with PBS.

6. RT-PCR analysis for TSLPR mRNA levels

To identify whether TSLP can act on iNKT cells, RT-PCR for TSLPR mRNA expression was performed. Total RNA was isolated with TRIzol reagent (Invitrogen). cDNA synthesis and gene amplifications were performed as described by the PCR kit protocol (Qiagen). The mixture was incubated at 95^{o}C for 15 minutes before the first cycle and extended at 72^{o}C for 10 minutes after all cycles were completed. PCR products were visualized on 2% agarose gels containing 5 μg/ml ethidiumbromide. Primer sequences for IL-7R and TSLPR together with annealing temperatures were listed in Table 1.

Table 1. Oligonucleotide primers used for gene expression analysis by RT-PCR

Target gene		Nucleotide sequence	Annealing temp (^{o}C)
IL-7R	Sense	actacaaacagcacgctgcc	55
	Anti-sense	ttcctgccttcctcactcct	
TSLPR	Sense	ctgacctgtcctacggggat	54
	Anti-sense	cctggaagttcccttggtgt	

7. Flow cytometric analysis of cytokine production after treatment of TSLP

1) Cytokine production in PBMCs after treatment of TSLP to PBMCs: Isolated PBMCs were incubated with TSLP for 24 hours. Cells were fixed with fix/perm solution (BD bioscience, San Jose, CA, USA) for 20 minutes and washed with perm/wash solution (BD bioscience). PBMCs were stained with anti-human Vα24 antibody (Abcam) for 30 minutes at 4^{o}C and stained with anti-human IL-4/IFN-γ antibodies (BD bioscience) for 30 minutes at 4^{o}C then analyzed by flow cytometric analysis.

2) Cytokine production in iNKT cells after treatment of TSLP to iNKT cells: iNKT cells were sorted by epic altra cell sorter (Beckman Coulter). The sorted iNKT cells were incubated with TSLP for 24 hours. Cells were stained with PE-conjugated anti-human IL-4 and IFN-γ antibodies (BD bioscience) and analyzed by flow cytometric analysis as mentioned above.

8. Statistical analysis

Intensity of TSLP and Vα24 immunohistochemical staining and the significance of population of iNKT cells in PBMCs of NC and AD were analyzed by using the Mann-Whitney U test and independent two samples t-test. The correlation of iNKT cell population with EASI score and serum IgE level was analyzed by using the correlation analysis.

III. RESULTS

1. iNKT cell population in PBMCs of AD patients

The population of iNKT cells in PBMCs of AD patients and NC was evaluated by flow cytometric analysis. The proportion of Vα24-positive cells was measured by the WINMDI program. Flow cytometric analysis showed that the population of iNKT cells in PBMCs of NC was 1.05%, and 2.3% in an AD patient (Fig. 1). The population of iNKT cell in PBMCs of AD patient was higher than in NC. Because of the limitation to gain the enough volume of blood sample from each patient with AD, 10 ml of blood sample was obtained from each patient and NC. Thirteen NC and sixteen AD patients were selected to measure the population of iNKT cells in PBMCs. As shown in Fig. 2, the population of iNKT cells in PBMCs of NC and AD patients was 0.6±0.3% and 1.7±0.4%, respectively. The number of iNKT cells in PBMCs of AD patients was statistically increased than those in PBMCs of NC ($p<0.001$).

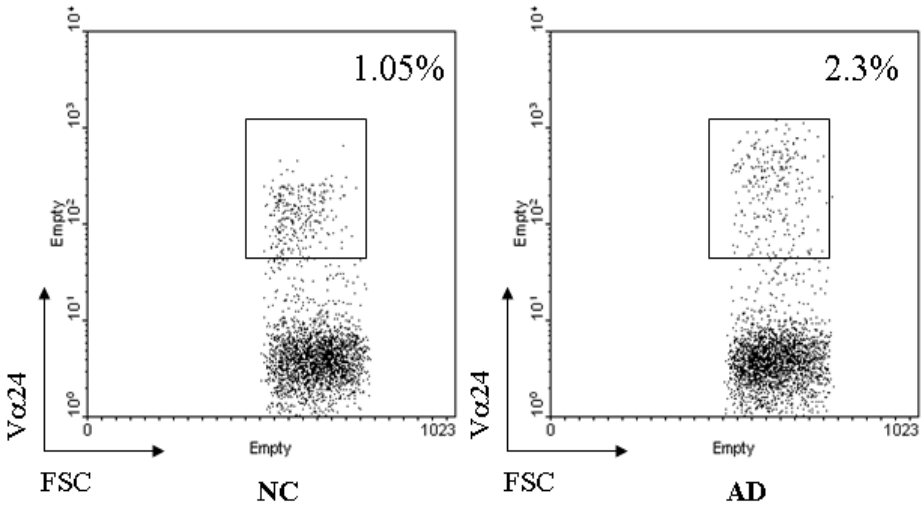


Fig. 1. Flow cytometric analysis of iNKT cell population in NC and in an AD patient. The population of iNKT cells in PBMCs of an AD patient was much higher compared with those in PBMCs of non-atopic NC.

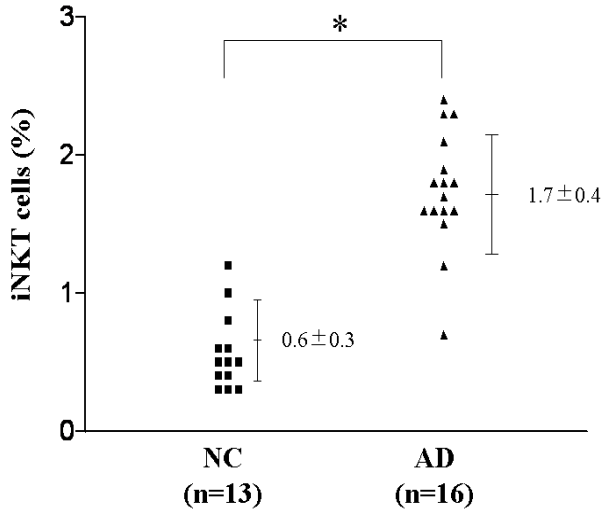
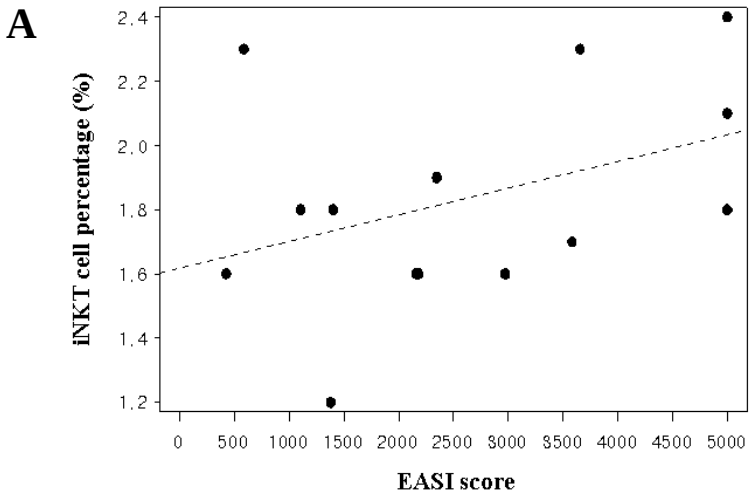


Fig. 2. iNKT cell population in PBMCs from NC and AD patients. PBMCs from 13 non-atopic NCs and 16 AD patients were analyzed by flow cytometry. The percentage of Vα24-positive iNKT cells in AD patients (1.7±0.4%) was higher than in non-atopic NCs (0.6±0.3%) ($p<0.001$).

2. Correlation of iNKT cell population and EASI score/serum IgE level in AD patients

To clarify whether the severity of AD patients and IgE secretion can relate with iNKT cell population, the correlation between the population of Vα24-positive cells and ESAI score or serum IgE level was examined by correlation analysis. Correlation coefficient r^2 value between EASI score and iNKT cell population was 0.1582, and r^2 value between IgE level and iNKT cell population was 0.0489. There was no correlation between iNKT cell population and EASI score (Fig. 3A, $p>0.05$) or serum IgE level (Fig. 3B, $p>0.05$).



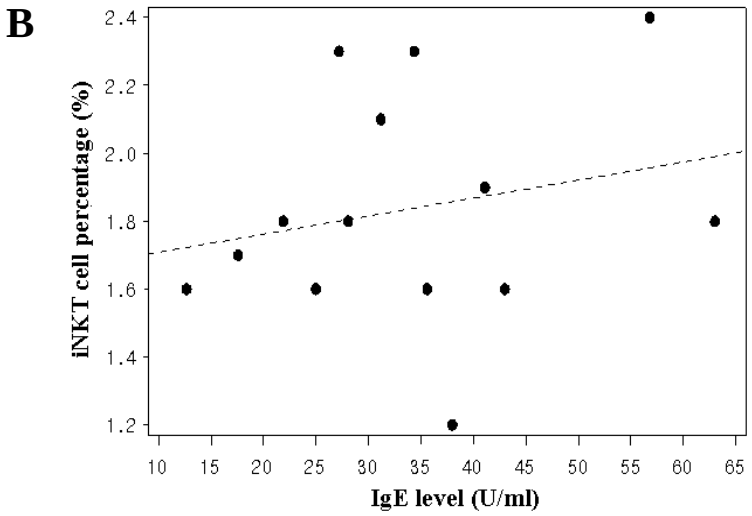


Fig. 3. Correlation of iNKT cell population and EASI score/serum IgE level. There was no significant correlation between the iNKT cell population and EASI score (A, r^2=0.1582, p>0.05) or serum IgE level (B, r^2=0.0489, p>0.05).

3. Effect of TSLP-treated DC on the population of iNKT cells in PBMCs

TSLP-activated DC from NC was co-cultured with PBMCs for 24 hours to clarify whether TSLP has an effect on iNKT cell population in PBMCs through DC activation. There was no significant difference in iNKT cell population between PBMCs co-cultured with TSLP-treated DC and PBMCs co-cultured with TSLP-untreated DC (Fig. 4).

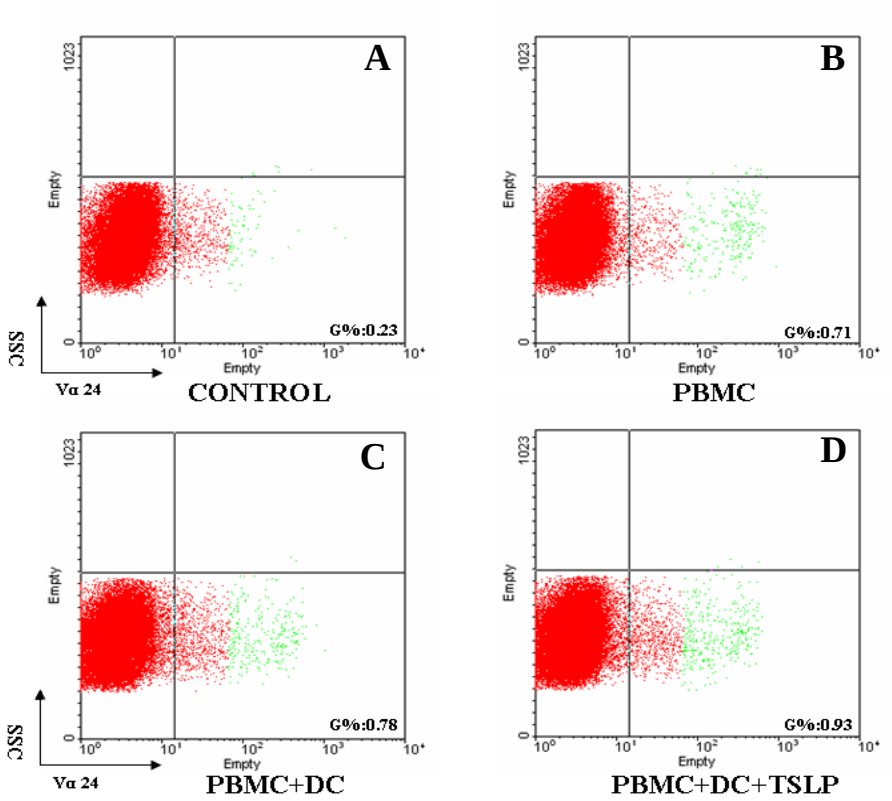


Fig. 4. Vα24-positive cell population in PBMC co-cultured with TSLP-activated DC. At day 6, immature DCs were incubated with 10 ng/ml TSLP for 24 hours. The PBMCs of NCs were divided into four groups. The TSLP-treated DCs and untreated DCs were co-cultured with each part of PBMCs for 24 hours. There were no significant differences in the iNKT cell percentage in four groups. Isotype control (A), PBMCs (B), PBMCs co-cultured with TSLP-untreated DCs (C), PBMCs co-cultured with TSLP-treated DCs (D).

4. TSLP expression in cultured human keratinocytes

To investigate TSLP expression in cytokine-treated and cytokine-untreated groups of cultured human keratinocytes, Western blot and RT-PCR of TSLP were performed. As shown in Fig. 5, cultured keratinocytes expressed TSLP; however IL-4, TNFα, transforming growth factor-β, and keratinocyte growth factor treatment did not change TSLP expression on both western blot and RT-PCR.

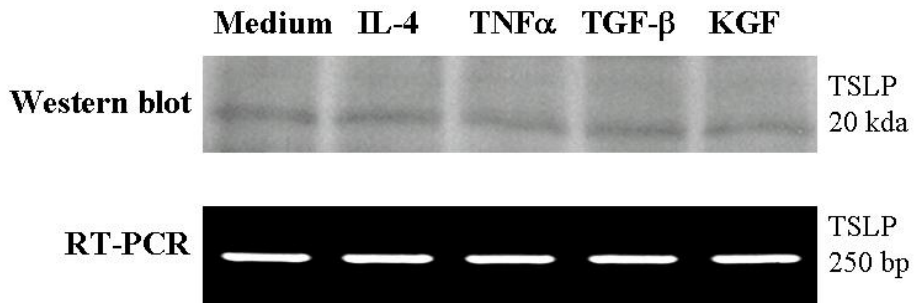


Fig. 5. Expression of TSLP protein and mRNA in cultured human keratinocytes. After treatment of 800 U/ml IL-4, 20 ng/ml TNF-α, 10 ng/ml TGF-β and 25 ng/ml KGF respectively, human keratinocytes were incubated for 16 hours. TSLP protein level was detected by western blot analysis; TSLP mRNA level was detected by RT-PCR analysis. Cytokine treatment did not change TSLP expression.

5. TSLP expression in the lesional skin of AD patients

To demonstrate the specificity of TSLP expression in the lesional skin of AD patients, skin biopsy samples from NC, patients with psoriasis and patients with AD were immunohistochemically stained with TSLP antibody. As shown in Fig. 6, non-atopic NC skin and psoriasis patient's skin expressed low quantitative TSLP (Fig. 6B, 6C) in both epidermis and dermis compared to isotype control (Fig. 6A); in contrast, TSLP was highly expressed in AD skin, mainly in epidermis (Fig. 6D). The skins from ten NC, three psoriasis patients and ten AD patients were stained to evaluate TSLP expression. The semiquantitative analysis focused on the intensity of the immunohistochemical reaction for TSLP. Color intensity was scored as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong) and the percentage of stained field in visual field was scored as 0 (0%), 1 (0~40%), 2 (40~80%), 3 (>80%)[41]. The color intensity score and occupied field score (from 0 to 6) were added to be final staining intensity and independently evaluated by two investigators[41]. As listed in Table 2, TSLP staining intensity in the epidermis of NC, psoriasis and AD skins were 1.42 ± 0.58, 1.87 ± 0.31 and 4.35 ± 0.43 respectively. TSLP expression was higher in patients with AD than in NC and patients with psoriasis mainly in epidermis ($p<0.001$).

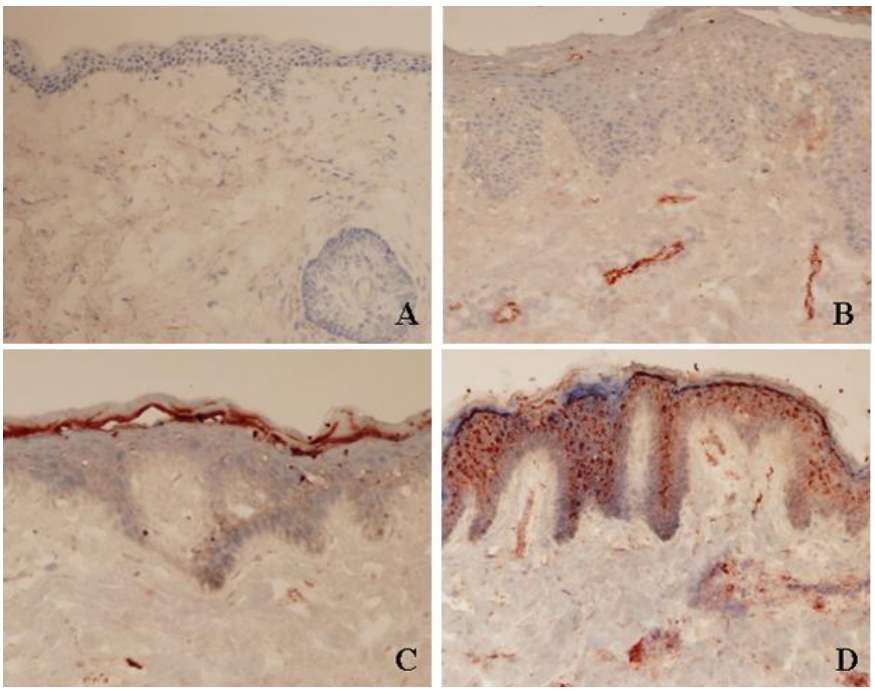


Fig. 6. Expression of TSLP in the skin of an AD patient. TSLP was mainly expressed in epidermis of AD skin. TSLP expression was detected in the epidermis of AD patient; non-atopic NC skin did not show TSLP expression. Negative staining with isotype control (A), a sample from one non-atopic NC (B), a lesion from one psoriasis patient (C), and a lesion from one AD patient (D).

Table 2. Mean expression of TSLP in the skin of NC, psoriasis patients and AD patients

Groups	Staining intensity	
	Epidermis	Dermis
Normal subjects (n=10)	1.42±0.58	1.06±0.33
Psoriasis patients (n=3)	1.87±0.31	0.68±0.83
Atopic dermatitis patients (n=10)	4.35±0.43	2.16±0.71
P value	<0.001	<0.001

Skin biopsy samples from ten NC, three psoriasis and AD patients were stained to evaluate TSLP expression. The intensity of the TSLP staining were independently evaluated by two investigators from low (0) to high (6) grade. *P<0.001

6. Expression of TSLP and Vα24 in the lesional skin of AD patients

To clarify whether both TSLP and Vα24-positive iNKT cells present in the lesional skin of AD patients, immunohistochemical staining with TSLP and Vα24 antibody was performed. The peroxidase-conjugated secondary antibody was used to detect TSLP (red) and alkaline phosphatase-conjugated secondary antibody was used to detect Vα24 (blue). TSLP was highly expressed in the epidermis (Fig. 7A, 7D) and Vα24 was highly expressed in

the dermis vessel of lesional skin of AD patients compared with NC skin (Fig. 7B, 7E). The double staining showed TSLP and Vα24 expressed in the NC and AD skin concurrently. TSLP staining intensity was evaluated as mentioned above. The number of Vα24 positive cells in high power field (x200) was scored as the intensity of the Vα24 stainings. 0 (negative) 1 (10 cells), 2 (20 cells), 3 (30 cells), 4 (40 cells), 5 (50 cells), 6 (more than 50 cells)[42]. The staining intensity was independently evaluated by two investigators. As shown in Table 3, TSLP staining intensity in epidermis was 1.72±0.28 and 3.35±0.39 in NC and AD patients; Vα24 staining intensity was 0.76±0.33 and 3.06±0.56 in NCs and AD patients' dermis. Both TSLP expression and Vα24-positive cells were increased in AD skins than in NC skins ($p<0.001$).

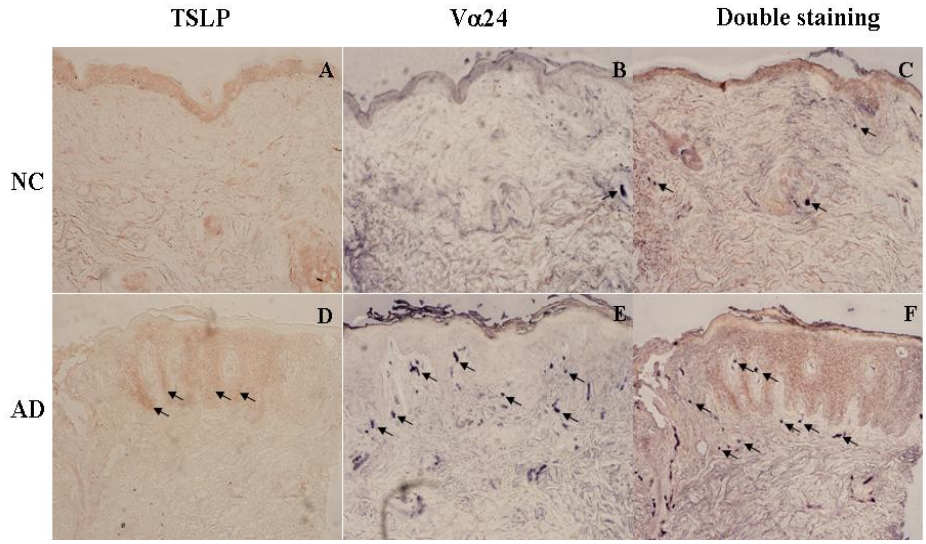


Fig. 7. Expression of TSLP and Vα24 in AD lesional skin. TSLP was mainly expressed in epidermis and Vα24 was expressed in dermis of skin biopsy samples from NC and AD patients. Both TSLP and Vα24 were highly expressed in AD skin. AD skin showed more intense expression of Vα24 than NC skin.

Table 3. Mean expression of TSLP and Va24 in double staining in the skin of NC and AD patients

	Staining intensity	
Groups	TSLP	Vα24
Normal subjects (n=5)	1.72±0.28	0.76±0.33
Atopic dermatitis patients (n=5)	3.35±0.39	3.06±0.56
P value	<0.001	<0.001

Skin biopsy samples from five NCs and five AD patients were double stained with anti-human TSLP antibody and anti-human Vα24 antibody to evaluate TSLP and Vα24 expression. The intensity of the TSLP and Vα24 staining were independently evaluated by two investigators from low (0) to high (6) grade. *P<0.001

7. Expression of TSLPR and IL-7R on the surface of iNKT cells

1) TSLPR protein expression in iNKT cells: As mentioned previously, TSLP can only act on cells that express TSLPRs. TSLPR expression was

measured by flow cytometric analysis to clarify whether TSLP has an effect on iNKT cells. As shown in Fig. 8, the TSLPR was highly expressed on the surface of Vα24-positive cells.

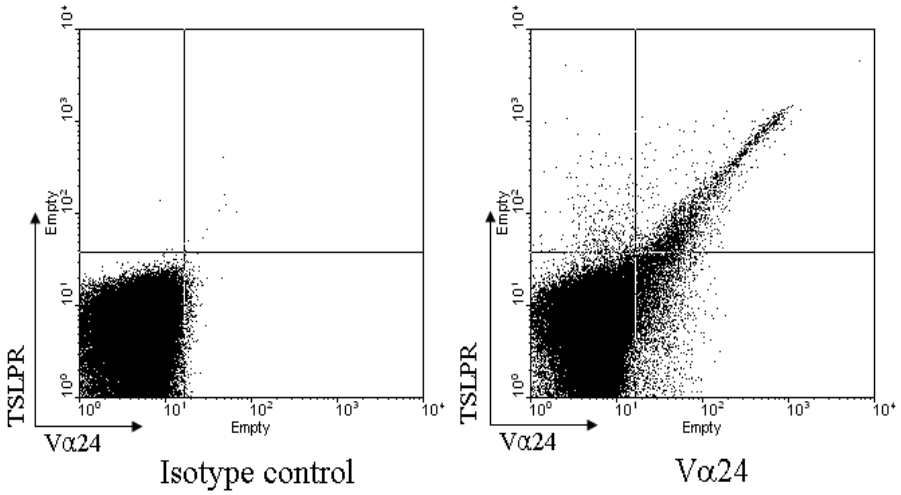


Fig. 8. Flow cytometric analysis of TSLPR expression in iNKT cells of healthy controls. PBMCs were stained by two-color fluorescences (Vα24-FITC/TSLPR-PE) and the cells were analyzed by flow cytometry. TSLPR was highly expressed on the surface of Vα24-positive cells.

2) TSLPR and IL-7R mRNA expression in iNKT cells: The $V\alpha24^+$ cells were isolated from PBMCs by the MACS system and the TSLPR mRNA and IL-7R mRNA level were analyzed by RT-PCR analysis. As shown in Fig. 9, both Vα24-positive and Vα24-negative cells expressed TSLPR and IL-7R.

However, TSLPR was highly expressed in Vα24-positive cells and IL-7R was highly expressed in Vα24-negative cells (Fig. 9).

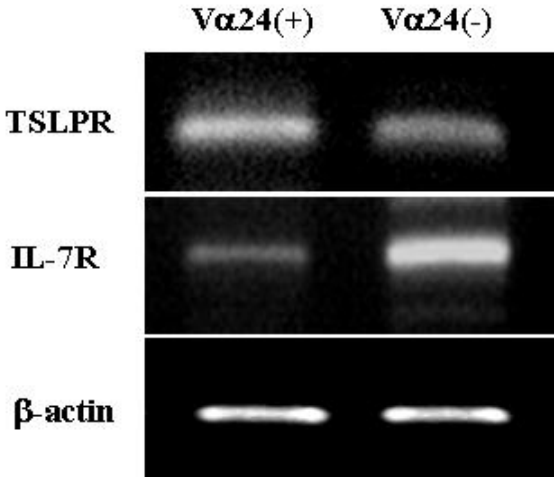


Fig. 9. RT-PCR analysis of TSLPR and IL-7R mRNA in Vα24-positive cells and Vα24-negative cells of NCs. mRNA of TSLPR and IL-7R was detected in both Vα24-positive and Vα24-negative cells. TSLPR was highly expressed in Vα24-positive cells and IL-7R was highly expressed in Vα24-negative cells.

8. Effects of TSLP on IL-4–secreting iNKT cells population in PBMCs

PBMCs of NCs were incubated with 10 ng/ml TSLP for 24 hours. The cells were stained by Vα24 antibody and incubated with PE-conjugated anti-human IL-4 antibody or PE-conjugated anti-human IFN-γ antibody for 30 minutes.

The percentage of IFN-γ–secreting iNKT cells in PBMC which was incubated with TSLP showed little increase than in PBMC which was cultured in media only. However, the percentage of IL-4–secreting iNKT cells was increased significantly after TSLP treatment (Fig. 10) than those of the percentage of IFN-γ–secreting iNKT cells in TSLP-treated PBMCs.

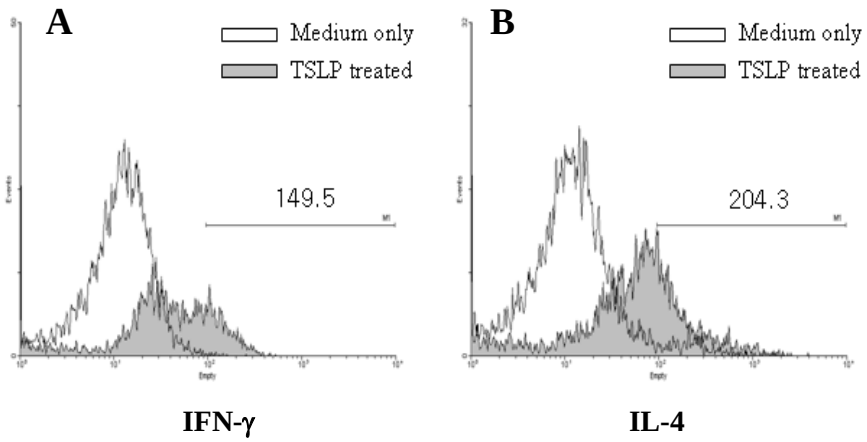


Fig. 10. Double-staining flow cytometirc analysis for Vα24 and IFN-γ or IL-4 in PBMCs of NCs treated with TSLP. Double-staining flow cytometric analysis was performed to analyze the effect of TSLP on the population of iNKT cells in PBMCs. IFN-γ expressing iNKT cells not show remarkable change (A), but IL-4–expressing iNKT cells increased remarkably (B) after 10 ng/ml of TSLP treatment .

9. Effects of TSLP on IL-4 and IFN-γ expression in iNKT cells

After iNKT cells of NC were incubated with 10 ng/ml of TSLP for 24 hours, the expression of IL-4 in iNKT cells was increased (Fig. 11A), but IFN-γ expression was not affected by TSLP treatment (Fig. 11B).

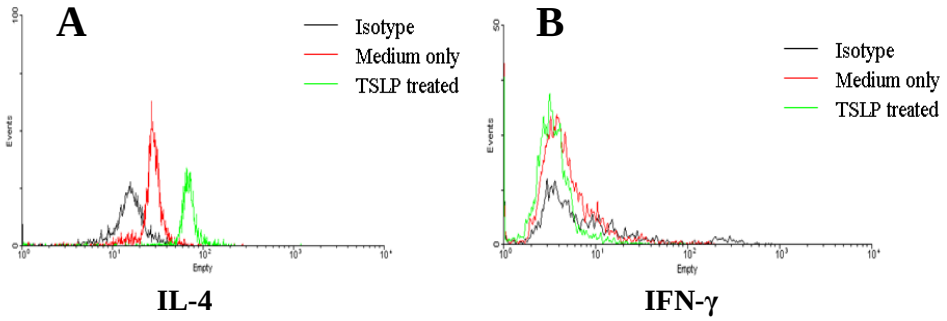


Fig. 11. Flow cytometirc analysis of IL-4 expression in iNKT cells of NC. To study the effect of TSLP on iNKT cells, iNKT cells were treated with 10 ng/ml of TSLP for 24 hours. IL-4 expression increased in the TSLP-treated iNKT cells (A), but IFN-γ expression was not affected by TSLP treatment (B).

IV. DISCUSSION

AD is developed by a complex interaction of various susceptibility genes, host environments, infectious agents, defects in skin barrier function, and immunologic responses[1-3]. Activation of T lymphocytes, DCs, macrophages, keratinocytes, mast cells, and eosinophils are characteristic of AD skin inflammatory responses[43]. As the biphasic TH1/TH2 switch in immune responses is important for the development of AD[41], it is necessary to control the TH1/TH2 balance for the treatment of AD. Recently, it has been reported that TSLP and iNKT cells are involved in asthma[44,45]. The aims of this study were to investigate the effect of TSLP on iNKT cells in healthy donors and AD patients, and to clarify whether TSLP and iNKT cells are involved in type 2 immune responses.

During the last decade, significant amount of data about the suppressive effects of regulatory T cells in models of autoimmunity, allergy, transplantation tolerance, tumor tolerance, and chronic infections have been suggested[44]. Regulatory T cells include naturally occurring CD4+CD25+ Tregs, TR1, TH3 and iNKT cells[47-49]. Although iNKT cells in these subsets are minor population cells, activated NKT cells produce large amounts of TH1-type and/or TH2-type cytokines, especially IFN-γ and IL-4 that lead to downstream activation of DCs, NK cells, B cells, and conventional T cells[42].

iNKT cells, which develop in the thymus, can induce both type 1 and type 2 immunity[23-26]. They are absent from nude mice, do not develop in thymectomized mice, and first appear in the thymus slightly later than conventional T cells.

The present data showed that the population of iNKT cells was increased in the PBMC of AD patients. The average iNKT cell population in 16 AD patients was 1.7 % in total PBMCs and 0.6 % in healthy donors. Because of iNKT cell is a minor population cell in PBMCs, to visualize the spot in flow cytometric analysis $1x10^5$ PBMCs were counted in one case. Then the population of iNKT cell in NC was higer than the mean population showed in Fig. 2. Although iNKT cell is a minor population cell in PBMCs, the population was increased significantly in AD patients. Because of the efficacy of iNKT cells, this result may be due to the iNKT cells expressing IL-4 and IFN-γ. This data does not indicate whether the effect of iNKT cells was positive or negative in type 2 immunity.

IgE levels are often raised in atopic disease, such as AD and asthma[50]. When assessing children or adults for the presence of atopic disease a raised level of IgE aids the diagnosis, although a normal IgE level does not exclude atopy[50]. The serum IgE was secreted by B cells and controlled by TH cells, especially affected by IL-4 secreting TH2 cells[50]. Inasmuch as iNKT cell not

only has immune suppressing functions, but also has immune accelerating functions, it's been hypothesized the increased population of iNKT cell may have relation with IgE level and the severity of AD. However, in the present study, there's no correlation between iNKT cell population with severity of AD and IgE level. According to EASI score was not related with iNKT cell population, the iNKT cells may not be a direct factor for the development of skin lesion of AD. There were several probabilities for this result. Firstly, the minority of iNKT cells in PBMCs and IL-4 secretion from iNKT cells may not enough to affect the B cells. Secondary, the number of cases included in this result was not enough to examine the correlation between the iNKT population with severity of AD and IgE level. More results are needed to evaluate the effect of iNKT cells in AD.

TSLP can act on DC to induce diverse immune reactions. When DC is treated with TSLP, DCs express several kinds of chemokines such as thymus and activation-regulated chemokine (TARC) and monocyte derived chemokine (MDC) that consequently induce TH2 immune response[15] However, in the present result, TSLP-treated DC had no effect on iNKT cell population. This data suggest that there may exist other factors to cause the increase of iNKT cell population.

In this study, the TSLP mRNA and protein were detected in cultured human

keratinocytes. IL-4 and TNFα are important cytokines to induce TH2 immune reaction and inflammation and KGF can induce TSLP expression from thymocyte. However, all these cytokines failed to regulate the TSLP secretion from cultured human keratinocytes. The result of immunohistological study showed in Fig. 6, expression of TSLP was not only detected in the epidermis of lesional skin of AD but also in the dermis. However, the squamous layers of the epidermis were mainly stained with TSLP. Soumelis and Liu[15] reported that TSLP expression was not detected in the epidermis or dermis of the NC skin and non-lesional skin of AD patients. In this study, there was an obvious tendency for TSLP expression in NC and psoriasis cases. Both epidermis and dermis expressed TSLP, although the expression intensity was lower than in AD cases. The result was conflicted with other reports. Also discrepancy between TSLP expression of *in vitro* cultured keratinocytes and those of *in vivo* skin need to further research.

TSLP acts on cells, which express TSLPR. The TSLPR complex consists of a TSLP binding chain and the IL-7R chain[37]. Epithelial cells appear to be the major producer of TSLP in both mice and human beings. Extensive analyses of TSLPR mRNA in highly purified primary cells, including all human immune cell types and cell lines, showed that cultured myeloid DC and plasmacytoid DC expess TSLPR, and cultured mast cells also have the

potential to express TSLPR[32-35]. It has been reported that TSLPR is expressed on mouse iNKT cells, but there's no report about expression of TSLPR on human iNKT cells. The results in Fig. 8 and Fig. 9 showed that TSLPR was expressed on Vα24+ iNKT cells. This result suggests that TSLP may directly act on iNKT cells and TSLP may have an effect on iNKT cells in AD.

In double staining for TSLP and Vα24 expression. The results showed that although no double stained spots were detected in the skin of AD patients, high expression of TSLP mainly in the epidermis and iNKT cells mainly in the dermis was observed in the skin of AD patients concurrently and the intensity of Vα24 staining in AD was also higher than in NC. This result was consistent with flow cytometric analysis of iNKT cell population in PBMCs of AD patients and immunohistochemical staining of TSLP in the lesional skin of AD patients.

In the present study, TSLP-treated PBMCs showed higher population of IL-4 secreting iNKT cells comparing with TSLP-untreated PBMCs. Furthermore, TSLP-treated iNKT cells showed high expression of IL-4, but IFN-γ expression was not significantly changed by TSLP treatment. This data suggests that TSLP can activate iNKT cells directly to produce IL-4, resulting in a TH2 immunologic effect.

In fact, activated iNKT cells are one of the potent immune modulators

acting through massive production of various cytokines including IL-4 and IFN-γ. They are also the major source of cytokines among TCR-nearing cells after stimulation with anti-CD3 antibody *in vivo*. And iNKT cells are like a double-edged sword as they can enhance or suppress a disease course depending on the immune condition of host[51]. This indicates that an increased population of iNKT cells might have an effect on the pathogenesis of AD. In addition, TSLP could activate not only DC but also iNKT cells to express type 2 cytokines such as IL-4 and IL-13. In contrast, the type 1 cytokine IFN-γ did not show any significant changes. These findings correspond to findings in AD which showed high expression of IL-4 and IL-13, but not IFN-γ.

However, further research will be needed to clarify the signaling of TSLP on iNKT cell activation and the discovery of TSLP antagonist may be helpful for AD therapy.

V. CONCLUSION

This study showed iNKT cells expressed TSLPR and TSLP directly activated iNKT cells to secrete IL-4 not via dendritic cells and the population of iNKT cells in PBMCs was increased in AD patients.

In conclusion, expression of TSLP in AD might activate iNKT cells to secrete TH2 cytokine IL-4 and thus activated iNKT cells might be involved in TH2 immune responses of AD.

REFERENCES

1. Leung DYM. Pathogenesis of atopic dermatitis. J Allergy Clin Immunol 1999;104:S99-108
2. Hanifin JM. Atopic dermatitis. J Allergy Clin Immunol 1984;73:211-26
3. Wollenberg A, Bieber T. Atopic dermatitis: from genes to skin lesions. Allergy 2000;55:205-13
4. Novak N, Bieber T. The skin as a target for allergic diseases. Allergy 2000;55:103-7
5. Hanifin JM. Critical evaluation of food and mite allergy in the management of atopic dermatitis. J Dermatol 1997;24:495-503
6. Kevin DC. Atopic dermatitis: recent trends in pathogenesis and therapy. J Invest Dermatol 1994;102:128-37
7. Coleman R, Trembath RC, Harper JI. Genetic studies of atopy and atopic dermatitis. Br J Dermatol 1997;136:1-5
8. Schaefer L, Kragballe K. Abnormalities in epidermal lipid metabolism in patients with atopic dermatitis. J Invest Dermatol 1991;96:10-5
9. Abeck D, Mempel M. *Staphylococcus aureus* colonization in atopic dermatitis and its therapeutic implications. Br J Dermatol

1998;139:13-6

10. Leung DYM, Sorter NA. Cellular and immunologic mechanisms in atopic dermatitis. J Am Acad Dermatol 2001;44:1-12
11. Jujo K, Renz H, Abe J, Gelfand EW, Leung DY. Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. J Allergy Clin Immunol 1992;90:323-31
12. Van Reijsen FC, Bruijnzeel-Koomen CA, Kalthoff FS, Maggi E, Romagnani S, Westland JK, et al. Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. J Allergy Clin Immunol 1992;90:184-93
13. Morren Ma, Przybilla B, Bamelis M, Heykants B, Reynaers A, Degreef H. Atopic dermatitis:triggering factors. J Am Acad Dermatol 1994;31:467-73
14. Biedermann T, Röcken M, Carballido JM. TH1 and TH2 lymphocyte development and regulation of TH cell-mediated immune responses of the skin. J Investig Dermatol Symp Proc 2004;9:5-14
15. Soumelis V, Liu YJ. Human thymic stromal lymphopoietin: a novel epithelial cell-derived cytokine and a potential key player in the induction of allergic inflammation. Springer Semin Immunopathol 2004;25:325-33

16. Sims JE, Williams DE, Morrissey PJ, Garka K, Foxworthe D, Price V, et al. Molecular cloning and biological characterization of a novel murine lymphoid growth factor. J Exp Med 2000;192:671-80
17. Tonozuka Y, Fujio K, Sugiyama T, Nosaka T, Hirai M, Kitamura T. Molecular cloning of a human novel type I cytokine receptor related to &/TSLPR. Cytogenet Cell Genet 2001;93:23-5
18. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature 1996;383:787-93
19. Bendelac A, Rivera MN, Park SH, Roark JH. Mouse CD1-specific NK1 T cells: development, specificity, and function. Annu Rev Immunol 1997;15:535-62
20. Kronenberg M. Toward an understanding of NKT cell biology: progress and paradoxes. Annu Rev Immunol 2005;23:877-900
21. Taniguchi M, Harada M, Kojo S, Nakayama T, Wakao H. The regulatory role of Vα14 NKT cells in innate and acquired immune response. Annu Rev Immunol 2003; 21:483-513
22. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? Nat Rev Immunol 2004;4:231-37
23. Akbari O, Stock P, Meyer E, Kronenberg M, Sidobre S, Nakayama T , et al. Essential role of NKT cells producing IL-4 and IL-13 in the

development of allergen-induced airway hyperreactivity. Nat Med 2003;9:582–8

24. Kawano T, Cui J, Koezuka Y, Motoki K, Ueno H, Nakagawa R, et al. CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides. Science 1997;278:1626-9
25. Chiba A, Oki S, Miyamoto K, Hashimoto H, Yamamura T, Miyake S. Suppression of collagen-induced arthritis by natural killer T cell activation with OCH, a sphingosinetruncated analog of alpha-galactosylceramide. Arthritis Rheum 2004;50:305-13
26. Ueno Y, Tanaka S, Sumii M, Tazuma S, Taniguchi M, Yamamura T, et al. Single dose of OCH improve mucosal T helper type 1/T helper type 2 cytokine balance and prevents experimental colitis in the presence of Vα14 natural killer T cells in mice. Inflamm Bowel Dis 2005;11:35-41
27. Miyake S, Yamamura T. Therapeutic potential of glycolipid ligands for natural killer (NK) T cells in the suppression of autoimmune diseases. Curr Drug Targets Immune Endocr Metabol Disord 2005;3:315-22
28. Chang YJ, Huang JR, Tsai YC, Hung JT, Wu D, Fujio M, et al. Potent immune-modulating and anticancer effects of NKT cell stimulatory

glycolipids. Proc Natl Acad Sci USA 2007;104:10299-304

29. Brigl M, Brenner MB. CD1: antigen presentation and T cell function. Annu Rev Immunol. 2004;22:817-90
30. Sims JE, Williams DE, Morrissey PJ, Garka K, Foxworthe D, Price V, et al. Molecular cloning and biological characterization of a novel murine lymphoid growth factor. J Exp Med 2000;192:671-80
31. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Liu YJ, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat Immunol 2002;3:673-80
32. Tonozuka, Y, Fujio K, Sugiyama T, Nosaka T, Hirai M, Kitamura T. Molecular cloning of a human novel type I cytokine receptor related to &/TSLPR. Cytogenet Cell Genet. 2001;93:23−5
33. Pandey A, Ozaki K, Baumann H, Levin SD, Puel A, Farr AG, et al. Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. Nature Immunol. 2000;1:59−64
34. Park LS, Martin U, Garka K, Gliniak B, Di Santo JP, Muller W, et al. Cloning of the murine thymic stromal lymphopoietin (TSLP) receptor: Formation of a functional heteromeric complex requires interleukin 7 receptor. J Exp Med 2000;192:659−70

35. Reche PA, Soumelis V, Gorman DM, Clifford T, Liu Mr , Travis M, et al. Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. J Immunol 2001;167:336−43
36. Wang YH, Liu YJ. OX40-OX40L interactions: a promising therapeutic target for allergic diseases. J Clin Invest 2007;117:3655-7
37. Liu YJ. Thymic stromal lymphopoietin and OX40 ligand pathway in the initiation of dendritic cell-mediated allergic inflammation. J Allergy Clin Immunol 2007;120:238-44
38. 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;178:6720-4
39. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980;92:44-7
40. Schmitt J, Langan S, Williams HC. What are the best outcome measurements for atopic eczema? A systematic review. J Allergy Clin Immunol 2007;120:1389-98
41. Oh SH, Lee JE, Suh H, Chang NS, Lee KH. Effect of laminin coated infection-preventive collagen membrane on wound healing in guinea pig. Korean J Invest Dermatol 2001;8:223-30

42. Mino N, Takenaka K, Sonobe M, Miyahara R, Yanagihara K, Otake Y, et al. Expression of tissue inhibitor of metalloproteinase-3 (TIMP-3) and its prognostic significance in resected non-small cell lung cancer. J Surg Oncol 2007;95:250-7
43. Akdis CA, Akdis M, Bieber T, Bindslev-Jensen C, Boguniewicz M, Eigenmann P, et al. Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. Allergy 2006;61:969-87
44. Oki S, Miyake S. Invariant natural killer T (iNKT) cells in asthma: a novel insight into the pathogenesis of asthma and the therapeutic implication of glycolipid ligands for allergic diseases. Allergol Int 2007;56:7-14
45. Nagata Y, Kamijuk H, Taniguchi M, Ziegler S, Seino K. Differential role of thymic stromal lymphopoietin in the induction of airway hyperreactivity and Th2 immune response in antigen-induced asthma with respect to natural killer T cell function. Int Arch Allergy Immunol 2007;144:305-14

46. Gershon RK. A disquisition on suppressor T cells. Transplant Rev 1975;26:170-85

47. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155:1151-64

48. Suri-Payer E, Amar AZ, Thornton AM, Shevach EM. CD4+CD25+T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J Immunol 1998;160:1212-8

49. Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J Exp Med 1998;188:287-96

50. Fitzpatrick TB, Irwin MF, Arthur ZE, Klauss W, Austen KF, Lowell AG et al. Dermatology in general medicine(5th ed.), McGraw-Hill, (NY), Frost Publisher,1999

51. Shimosaka A. Role of NKT cells and alpha-galatosyl ceramide. Int J Hematol 2002;76:277-9

국문요약

아토피피부염에서 thymic stromal lymphopoietin이 invariant natural killer T cell에 미치는 영향

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Thymic stromal lymphopoietin (TSLP)은 아토피피부염의 발병에 중요한 역할을 하는 사이토카인으로 아토피피부염 환자의 표피에서 많이 발현된다. 아토피피부염에서 TSLP의 발현이 수지상세포에 미치는 영향에 대해서는 많은 연구가 이루어 졌지만 invariant natural killer T (iNKT) 세포에 미치는 영향에 대해서는 아직 연구가 미비한 실정이다. iNKT 세포는 regulatory T 세포의 특징을 보이며 활성화된 iNKT 세포는 interferon-γ (IFN-γ), interlukin-4 (IL-4)를 분비하여 면역반응을 조절한다.

본 연구에서 정상인과 아토피피부염 환자의 말초혈액에서 iNKT 세포의 비율을 비교분석하고 배양한 각질형성세포와 아토피피부염 환자의 병변 피부조직에서 TSLP의 발현을 관찰하며, iNKT 세포에 TSLP를 처치한 후 iNKT 세포에서 생성되는 사이토카인을 분석하여 아토피피부염에서 TSLP가 iNKT 세포에 미치는 영향을 관찰하

고자 하였다.

본 연구에서 아토피피부염 환자의 말초혈액에서 정상인에 비해 iNKT 세포의 비율이 유의하게 증가되어 있음을 관찰하였다. iNKT 세포의 수는 아토피피부염 환자의 중증도 및 혈청 IgE 수치와는 통계학적으로 상관성이 없었으며 TSLP를 처치한 수지상세포를 말초혈액 단핵구 (PBMC)에 함께 배양한 경우 PBMC에서 iNKT 세포의 증가가 관찰되지 않았다. 배양한 각질형성세포에서 TSLP의 발현이 뚜렷이 관찰되였으나 IL-4, tumor necrosis factor α, transforming growth factor β, keratinocyte growth factor 처치 후에 각질형성세포에서 TSLP의 발현에 유의한 차이를 관찰할 수 없었다. 정상 피부조직 및 건선환자의 피부조직에 비해 아토피피부염 환자의 병변 피부조직에서 TSLP의 발현이 유의하게 증가되어 있음을 관찰하였으며 주로 표피에서 발현이 증가되어 있었다. 면역형광유세포분석과 RT-PCR 검사에서 iNKT 세포는 TSLP 수용체를 발현하였다. TSLP를 PBMC에 처리하였을 때 PBMC에서 IL-4를 분비하는 iNKT 세포의 수가 증가되었으며 iNKT 세포에 TSLP를 처리하였을 때 iNKT 세포에서 IL-4의 발현이 증가하였다. 이 결과는 TSLP가 수지상세포를 활성화시킬 뿐만 아니라 iNKT 세포에 직접적으로 작용하여 TH2 사이토카인을 분비하는 작용을 할 수 있음을 보여준다. 반면에 TH1 사이토카인인 IFN-γ 는 TSLP처리에도 발현의 차이를 보이지 않았다. 이러한 결과들은 아토피피부염환자에서 IFN-γ은 변화를 보이지 않으면서 IL-4, IL-13을 발현하는 세포가 증가하는 점과 일치하는 결과이다.

결과적으로 각질형성세포에서 발현되는 TSLP는 직접적으로 iNKT 세포를 자극하여 TH2 사이토카인인 IL-4의 분비를 자극하므로써 활성화된 iNKT 세포가 아토피피부염의 발병과 관련된 TH2형 면역반응과 밀접히 연관되어 있음을 알 수 있었다.

핵심되는말: 아토피피부염, thymic stromal lymphopoietin, invariant natural killer T cell