Role of interleukin-17 production of fatty acid binding protein 5-expressing CD4+ T cells in atopic dermatitis showing systemic involvement

Jungsoo Lee

Department of Medicine

The Graduate School, Yonsei University

Role of interleukin-17 production of fatty acid binding protein 5-expressing CD4+ T cells in atopic dermatitis showing systemic involvement

Directed by Professor Kwang Hoon Lee

Doctoral Dissertation
submitted to the Department of Medicine
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Jungsoo Lee

June 2017

연세대학교
YONSEI UNIVERSITY

This certifies that the Doctoral Dissertation of Jungsoo Lee is approved.

Thesis Supervisor : Kwang Hoon Lee

Thesis Committee Member#1 : In-Hong Choi

Thesis Committee Member#2 : Min-Geol Lee

Thesis Committee Member#3 : Jung-Won Park

Thesis Committee Member#4 : Jin Young Kim

The Graduate School
Yonsei University

June 2017

ACKNOWLEDGEMENTS

First of all, I would like to show my deepest gratitude to my supervisor, Professor Kwang Hoon Lee, M.D., Ph.D. for the full support and genuinely excellent guidance in order to successively finish my doctoral degree. He has been the one who inspired me the most as a dermatology researcher at all times and without his persistent help, this dissertation would not have been possible.

I would also like to thank my committee chair, Professor In-Hong Choi and committee members, Professor Min-Geol Lee, Professor Jung-Won Park and Doctor Jin Young Kim. Their valuable advices on my dissertation by sparing enormous precious time conveyed strength to my research.

In addition, I would like to express my appreciation to Professor Chang Ook Park for giving me the great scientific ideas and detailed academic advices. I am also grateful to my laboratory colleagues; Doctor Ji Yeon Noh for giving a major help to choose the target protein, Ji Hye Kim, Hyeran Kim, Seo Hyeong Kim, and Bo-Mi Kim for their time and efforts to help me with my experiments.

For the continuous encouragement and advices, I would like to thank Professor Jung U Shin and my resident colleagues Hong Sun Jang and Min Ju Choi. Also, thank you so much for your help, Howard Chu. And I'm truly thankful to Professor Lars E. French, who is a Chairman of Department of Dermatology, Zurich University Hospital, for the warm support and providing motivations to keep up my scientific work.

I am sending a special thanks to my dearest family; father and mother, you were always there for me throughout my entire life, supporting me with infinite love and sacrifice. Paul, you are the best and I truly, deeply love you with all of my heart. And my adorable daughter Yegyo, I love you so much! Not only as my precious family, but also as a great medical doctor and an admirable leader, a former Speaker of the National Assembly, I sincerely respect you Ui Hwa Chung, my father-in-law. Thank you for your warm-hearted support and love, my mother-in-law. Love you sister! You've always been brighter than me.

And last but not least, I thank God from the beginning till the end, for giving me a fundamental strength and courage to go on and for everything he has done for me. I dedicate my dissertation to you.

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLE

ABSTRACT

Role of interleukin-17 production of fatty acid binding protein 5-expressing CD4+ T cells in atopic dermatitis showing systemic involvement

Jungsoo Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Kwang Hoon Lee)

Evidences that support the recent increase of systemic atopic dermatitis (SAD) patients related with other inflammatory conditions are growing. The most definite is the so-called “atopic march (AM).” It is a natural history of atopic manifestations, showing a sequence of progression of clinical signs of atopic disease. But still, the mechanism underlying the development of SAD is not fully understood and many researchers are exploring to find the exact mechanism to explain the pathogenesis of SAD including AM. Thus, to find a possible mechanism of SAD, we designed a study in search of immune signatures and specific biomarker for SAD.

We developed murine models of pure atopic dermatitis (PAD) by skin sensitization only, and both skin and lung sensitization mimicking SAD patients respectively by using NC/Nga mice. Then, we performed RNA microarray, quantitative real-time PCR (qRT-PCR) and immunofluorescence staining using PAD and SAD human skin tissues and T cells.

We found that SAD displayed higher IL-17 production in lung and lymph nodes by flow cytometry analysis, which was further validated by qRT-PCR using PAD and SAD skin. We focused on fatty acid binding

protein 5 (FABP5), which was significantly expressed in SAD human skin in the RNA microarray analysis. We further confirmed the result in mouse and human SAD skin compared with PAD skin using qRT-PCR and confocal microscopy. Finally, we observed that CD4+ CD69+ T_{RM} (tissue-resident memory T) cells and CD4+ IL-17+ T cells in the human SAD skin had expressed FABP4/5 significantly than those cells in PAD skin.

Taken together, IL-17-mediated cutaneous inflammation of FABP5+ CD4+ T cells in SAD may explain the possible mechanism. Further study will be necessary to see the function of FABP5 in SAD.

Key words: systemic atopic dermatitis, fatty acid binding protein 5, IL-17, T_{RM} cells

Role of interleukin-17 production of fatty acid binding protein 5-expressing CD4+ T cells in atopic dermatitis showing systemic involvement

Jungsoo Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Kwang Hoon Lee)

I. INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease with severe itching sensation, followed by disturbances of quality of life.[1] There are many clinical evidences that support the idea of AD as a systemic disease. Descriptions of eye involvement[2-3], gastrointestinal involvement[4-5], renal involvement[6-7] and autoimmune disease[8-11] had been reported, but the most explicit evidence that had been illustrated was a phenomenon called 'atopic march'.[12]

Atopy is defined as a familial or personal propensity to produce IgE antibodies, sensitization to inhalant and food allergens[13,14] and underlying atopy has been considered to be critical in linking AD, allergic rhinitis and allergic asthma.[15,16] In its classic description, AD composes only a part of the atopic complex which comprises beyond skin changes and usually combines with airway involvement including lung and nasal mucosa, which explains the progression from atopic eczema to respiratory allergic diseases such as allergic rhinitis and allergic asthma.[15-17] These conditions can be unrelated disorders that sequentially develop along an atopic pathway or there might be a causal link between early eczematous skin lesion and the later-onset respiratory disorders.[17] However, the concept of the atopic march which represents AD as a systemic disease has been supported by cross-sectional and longitudinal studies.[18-23]

Currently, there are studies trying to explore the pathogenesis of systemic

atopic dermatitis (SAD), emphasizing the relationship between AD and onset of other respiratory allergic diseases; defective skin barrier during childhood eczema opens a way for epidermal water loss, and entry of high molecular weight allergens, bacteria, and viruses leads to eventual onset of airway hyperresponsiveness.[24] In order to explain the proposed molecular mechanism for the epicutaneous sensitization that promotes AM which represents SAD, several studies have provided evidence that T cells are essential for inflammation and airway sensitivity[25-27] and suggested that the epicutaneous entry of environmental allergens through barrier-disrupted skin inducing thymic stromal lymphopoietin (TSLP) expression is strongly associated with the induction of Type 2 helper T cells (Th2)-dominant immunologic responses followed by sensitized airways and airway inflammation.[28-29]

Pacciani V *et al.* characterized *in vitro* T-cell responses to group 2 major allergen of *Dermatophagoides pteronyssinus* (Der p 2), which was known to be one of the most potent allergens in AM.[30] As a result, Der p 2 specific Th2 response showed significant correlation with both clinical and immunologic parameters in SAD group and higher levels of T-cell proliferation was observed in SAD group when compared with NL (normal) group.

While other studies were focused on Th2 responses in SAD, an experiment using allergic asthma mouse model was performed.[31] Since genome-wide association study identified a significant association between a single nucleotide polymorphism in the IL-6 receptor gene in allergic asthma, the authors pitches into the IL-6 signaling pathway and related immunologic reactions. The experiment confirmed that allergen-induced IL-6 trans-signaling activated γδ T cells to promote Th2 and Th17 airway inflammation, offering additional information of Th17 response and its possible relationship with SAD.

Current genome-wide association study done by many researchers provided the relationship between IL-6 polymorphism and AD[32] but IL-17 polymorphism was never reported. However, Th17 differentiation stimulated by IL-6 and other cytokines, and the production of a plethora of cytokines, including IL-17 regulate local and systemic inflammation with vasculitis had been well-illustrated.[33]

In our previous study, we observed phenotypes in SAD patients and pure AD (PAD) patients at a single point in time since the characterization of SAD patients who retain AD beyond childhood has not been fully explored before (Table 1).[34] Comparison of skin lesion severity with Eczema Area and Severity Index, serum total IgE, and presence of family history between the two groups revealed no evident differences. There was no difference in the level of serum eosinophil as well. This finding displayed no remarkable phenotype difference between those two groups based on clinical characteristics. The only apparent difference between SAD patients to PAD patients was a presence of "greater" degree of sensitization to *Dermatophagoides farinae* (*D. farinae*).

Table 1. Comparisons of clinical and laboratory characteristics between PAD and SAD patients

	Serum specific IgE		Skin prick test	
	PAD	SAD	PAD	SAD
Male	22	27	20	28
Female	14	16	32	22
Total (n)	36	43	52	50
Age	23.88 ± 6.25	24.11 ± 6.69	19.9 ± 10.17	18.44 ± 6.70
EASI score	19.62 ± 10.62	16.19 ± 8.49	11.56 ± 7.53	11.98 ± 7.85
Total IgE	1963.91 ± 1930.09	1382.83 ± 1456.36	583.06 ± 696.14	927.36 ± 1026.413
WBC count (x1000)	7.02 ± 1.77	6.87 ± 1.60	6.85 ± 1.58	7.12 ± 1.58
Eosinophil (%)	6.11 ± 3.59	6.73 ± 3.52	4.72 ± 2.91	5.51 ± 2.98
D. farinae	83.3% (+)	90.7% (+)*	45.3% (+)	68% (+)*

* $p < 0.05$ when compared with PAD group.

Abbreviations: *EASI*, Eczema Area and Severity Index

Overall, since there are only a few clues of immune responses in SAD and as an exact mechanism underlying the development of SAD still debatable, we determined to explore the immune signatures and biomarkers for SAD. In the present study, we investigated immunological mechanisms for SAD by using SAD NC/Nga mouse model and human skin.

II. MATERIALS AND METHODS

1. Blood samples

Peripheral blood monocytes from healthy donors, PAD and SAD patients were isolated using Ficoll-Hypaque technique.

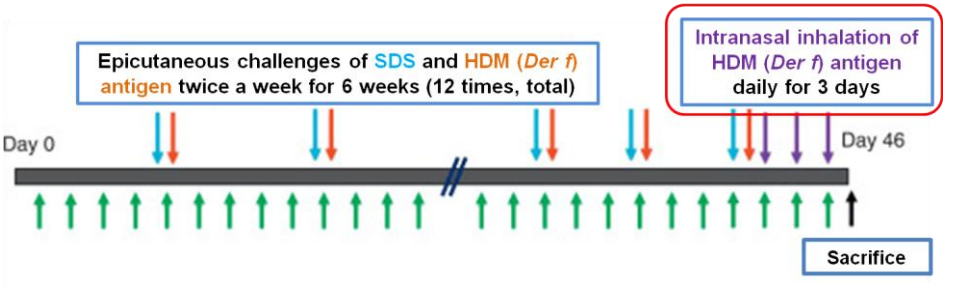
2. Experimental animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of Department of Laboratory Animal Resources, Yonsei Biomedical Research Institute, Yonsei University College of Medicine. Seventeen female NC/Nga mice (6 weeks old) were purchased from SLC Japan (Shizuoka, Japan). Mice were kept under controlled humidity (40%) and temperature (22±2℃) conditions. House dust mite (HDM) ointment was purchased from the Biostir Inc. (Kobe, Japan). One g of ointment contains 234 μg of group 1 major allergen of *D. farinae* (Der f 1), 7 μg of Der f 2 and 134.4 mg of other proteins.

3. Development of SAD model in NC/Nga mice

A total of seventeen NC/Nga mice was used in this experiment. Four mice were used as normal control, 5 mice were used to induce skin lesion only and 8 mice were used to develop the SAD model. On the first week, all of NC/Nga mice were anesthetized by intra-abdominal injection of zoletil (30-40 mg/kg) and the hair on their dorsal skin was shaved and completely removed by using hair removal cream and razors once a week. To induce skin inflammation, we applied 200 μl of 4% sodium dodecyl sulfate on PAD and SAD murine models' back and posterior auricular area to disrupt the skin barrier. After 2 hours, 100 μg HDM ointment was applied. Epicutaneous challenges of SDS and HDM were done twice a week for 6 weeks (a total of 12 applications). During the last 3 days of the experiment, to induce SAD murine model, 100 μg of *D. farinae* antigen was given daily by intranasal inhalation (Fig. 1). Twenty-four hours after the last challenge, specimens of skin, blood, lymph nodes, spleen and lung were collected to evaluate the immune responses. Animal experiments were done with the approval of the Research Ethics Committee of Severance Hospital.

(A)



(B)

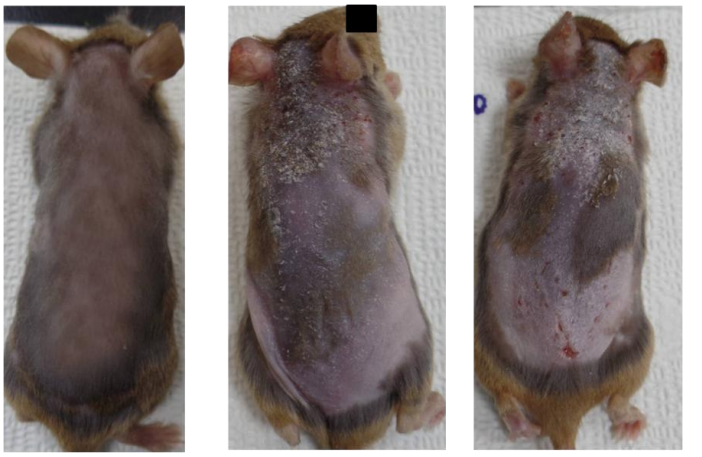


Figure 1. Development of PAD and SAD mouse models using NC/Nga mice. (A) To induce skin inflammation in both PAD and SAD murine models, SDS was applied two hours prior to 100 μg HDM (*D. fariane*) antigen topical application in order to disrupt the skin barrier. Epicutaneous challenges of HDM antigen were done twice a week for 6 weeks. To develop SAD models with respiratory allergic inflammation, additional intranasal inhalations of the 100 μg HDM were done for the last 3 days of the experiment. (B) Clinical photographs of NL, PAD and SAD mouse model after 6 weeks of the challenge. Normal skin was observed in the NL group while PAD and SAD group displayed eczematous skin lesion with erosion and scales.

Abbreviations: *NL*, normal; *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis; *SDS*, sodium dodecyl sulfate; *HDM*, house dust mite

4. Flow cytometric analysis

Cells were isolated from skin, lymph nodes, spleen and lung from murine models to prepare single cell suspensions from each tissue. For T cell activation, single cells were cultured in the anti-CD3 (eBioscience, Waltham, MA, USA) and anti-CD28 (eBioscience) coated plates. Next, cells were treated with Cell Stimulation Cocktail (plus protein transport inhibitors) (eBioscience) for 16 hours and then stained by using fixable viability dye for 30 min at 4℃. After washing, cells were labeled with anti-CD4 (eBioscience) and anti-CD-25 antibodies (eBioscience) conjugated with fluorescent dye (eBioscience). To perform intracellular labeling, cells were fixed and permeabilized with a cytofix/cytoperm buffer (eBioscience) and labeled with anti-mouse IL-4 (eBioscience), IL-10 (eBioscience), IL-17 (eBioscience), interferon-γ (IFN-γ) (eBioscience) and Foxp3 (eBioscience) conjugated with fluorescent dye. Labeled cell quantification was done by using a BD LSR Fortessa^{TM} flow cytometer. Flow Jo Software (BD Bioscience, San Jose, CA, USA) was used for data analysis.

5. Measurement of immunoglobulins, and cytokines in sera by enzyme-linked immunosorbent assay (ELISA)

Mouse sera were obtained from cardiac puncture. Immunoglobulin concentration was determined with a mouse IgE ELISA kit (BioLegend, San Diego, CA, USA) according to the manufacturer's instructions. IL-4 (BioLegend) ELISA was done.

6. Quantitative real-time PCR analysis

Skin samples from mice were homogenized by using a Precellys 24 Homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). For the total RNA extraction, RNeasy Plus Mini kit (Qiagen, Hilden, Germany) was used following the manufacturer's instructions and then subjected to a reverse transcription. At least two times of quantitative real-time PCR (qRT-PCR) were done for each sample using 2 μl of cDNA, supplemented with appropriate primers (Applied Biosystems, Foster City, CA, USA). Primers specific for mice -

IL-4 (Mm00445259_m1), IFN-γ (Mm01168134_m1), Foxp3 (Mm00475162_m1), IL-10 (Mm01288386_m1), IL-17a (Mm00439618_m1), FABP4 (Mm00445878_m1), FABP5 (Mm00783731_s1) and GAPDH (Mm99999915_g1) - were mixed with cDNA and qPCR master mix (Applied Biosystems) in 20 μl reaction. To perform qRT-PCR, StepOnePlus PCR system (Applied Biosystems) was used. The $2^{-\Delta\Delta C}{}_{T}$ method was used to calculate expression levels of mRNA. Additional qRT-PCR was done using *D. farinae* treated T cell for cDNA synthesis, 100 ng of total RNA was used and FABP5 primer (Qiagen) was supplemented for qRT-PCR.

7. Microarray analysis

All microarray analysis was performed at ProteomeTech Inc. in Yonsei University. RNA integrity and purity were evaluated by ND-1000 Spectrophotometer (NanoDrop, Wilmington, DE, USA), Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Total RNA was amplified and purified by using TargetAmp-Nano Labeling Kit for Illumina Expression BeadChip (Epicentre, Madison, WI, USA) for biotinylated cRNA according to the manufacturer's instructions. Four hundred ng of total RNA were reverse-transcribed using a T7-oligo (dT) promoter primer. After second-strand cDNA systhesis and purification, the cRNA quantification was done using the ND-1000 Spectrophotometer (NanoDrop). Seven hundred fifty ng of labeled cRNA samples were hybridized to each Human HT-12 v4.0 Expression Beadchip for 17 hours at 58℃, according to the manufacturer's instructions (Illumina, Inc., San Diego, CA, USA). Detection of array signal was performed using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK) following the bead array-manual. Arrays were scanned with an Illumina bead array Reader confocal scanner according to the manufacturer's instructions. Raw data extraction was carried out using the software provided by the manufacturer (Illumina GenomeStudio v2011.1 (Gene Expression Module v1.9.0)). Array probes were transformed by the logarithm and normalized by quantile method. The statistical significance of the expression data was determined using fold

change.

8. Tissue preparation and immunofluorescence staining

Tissue samples were routinely fixed with formalin and processed to paraffin blocks. Six μm sections were cut, mounted on slides and dried, deparaffinized in xylene and ethanol. For antigen retrieval, slides were boiled for 20 min in 10 mM sodium citrate buffer (pH 6.0) and cooled for 30 min at room temperature. Overnight incubation of sections at 4℃ was performed for triple immunofluorescence with a mixture of primary antibodies in a humid chamber. Sections were rinsed for 10 min in PBS and incubated for 1 h at room temperature with a mixture of secondary antibodies in a humid chamber. Sections were mounted in VECTASHILED mounting media and images were acquired by laser scanning confocal microscopy (LSM 780; Zeiss, Oberkochen, Germany). The antibody lists are described as follows; Primary antibody lists; CD4: bs-0647R-A555 (Bioss, Alexa 555 conjugated, 1:100), FABP4: ab93945 (abcam, 1:200), FABP5: ab84028 (abcam, 5 ug/ml), Secondary antibody lists; Anti-mouse Alexa.633 (A21070, ThermoFisher scientific, Waltham, MA, USA, 1:500) for FABP4, Anti-rabbit Alexa.633 (A21126, ThermoFisher scientific, 1:500) for FABP5.

9. Statistical analysis

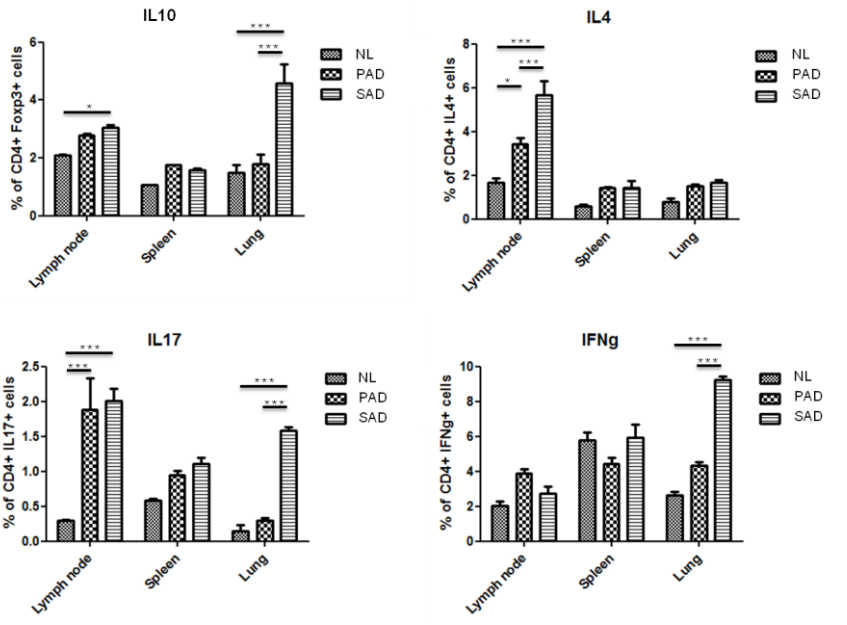
Statistical analysis was conducted using SPSS version 20 software (SPSS, Inc, Chicago, IL, USA). Statistical differences were considered to be significant at a value $p < 0.05$ and the correlation of nonparametric paired data was tested using Spearman's rho, and the significance was evaluated using the t-tests and analysis of variance (ANOVA) statistics with Bonferroni's multiple comparison test. Graphs were expressed as mean ± SEM using Prism software (Graphpad software, San Diego, CA, USA).

III. RESULTS

1. Expression of IL-17 in NL, PAD and SAD mouse model

The SAD group displayed statistically higher IL-17 in SAD mouse model by flow cytometry analysis, especially from lymph nodes and lung tissue. In spleen, even though the result was not significant, the same trend was observed. Similar results were observed for IL-4, but the result was only significant in lymph nodes (Fig. 2A). IL-10 expression was significant in lymph nodes and lung of SAD. For IFN-γ, the same result was only exhibited in the lung, with a marked expression in SAD. To extend these observations, further validation was performed by qRT-PCR using PAD and SAD skin (Fig. 2B). Pronounced gene expression of IL-17 in SAD skin than in PAD was confirmed with statistical significance, and IL-4 expression was also significantly increased by a lesser degree.

(A)



(B)

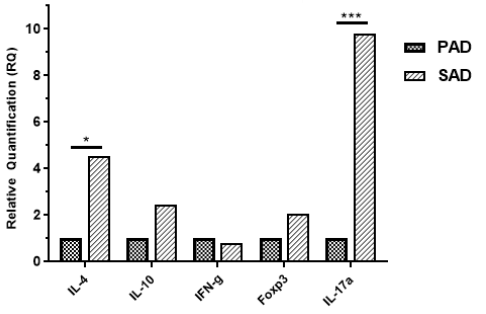


Figure 2. Results of flow cytometry analysis of CD4+ T cells and qRT-PCR of mouse models. (A) Flow cytometry analysis of lymph nodes, spleen, and lung were performed. Higher expression of IL-17 in CD4+ T cells was detected from the SAD mouse model with statistical significance in lymph nodes and lung. IL-10, which has a function as a counterpart of IL-17 has demonstrated similar results in lymph nodes and lung. For IL-4, its expression was significant in the lymph nodes of SAD and IFN-γ expression was significant only in the lung of SAD. (B) Significantly higher level of IL-17A gene expression was exhibited by QRT-PCR of SAD mouse skin. Significant expression of IL-4 genes, with a lesser degree was also observed in SAD. *$p < 0.05$, ***$p < 0.001$.

Abbreviations: *NL*, normal; *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis

2. The RNA microarray result reveals up-regulation of IL-17 cytokine family and fatty acid binding protein (FABP) 5 in human SAD skin

Microarray analysis using human samples exhibited distinguishable gene expression patterns in NL, SAD and PAD group (Fig. 3). Also, up regulation of IL-17 cytokine family, including IL-17a and IL-17f in SAD were demonstrated when compared with PAD and NL samples although statistical significance was not observed. Fold changes of IL-6, IL-21 and TGF-β1, which are related with IL-17 mediated inflammation, were also increased in SAD group (Fig. 4).

In search of a candidate biomarker protein, we selected the genes with significant fold changes. Among approximately 100 genes displaying at least two-fold up or down, as IL-17 was related to systemic inflammation in fat-rich environments, including skin, we screened for proteins involved in fatty acid metabolism and discovered a set of genes demonstrating significant fold changes. According to data analysis, fatty acid binding protein (FABP) family, including FABP5 and FABP5-like protein 2 (FABP5L2) showed significant fold increase and FABP4 and FABP9 displayed significant fold decrease (Fig. 5). Among these FABPs, FABP5 was selected as a candidate biomarker protein as it was an epidermal FABP, directly related to skin inflammation.

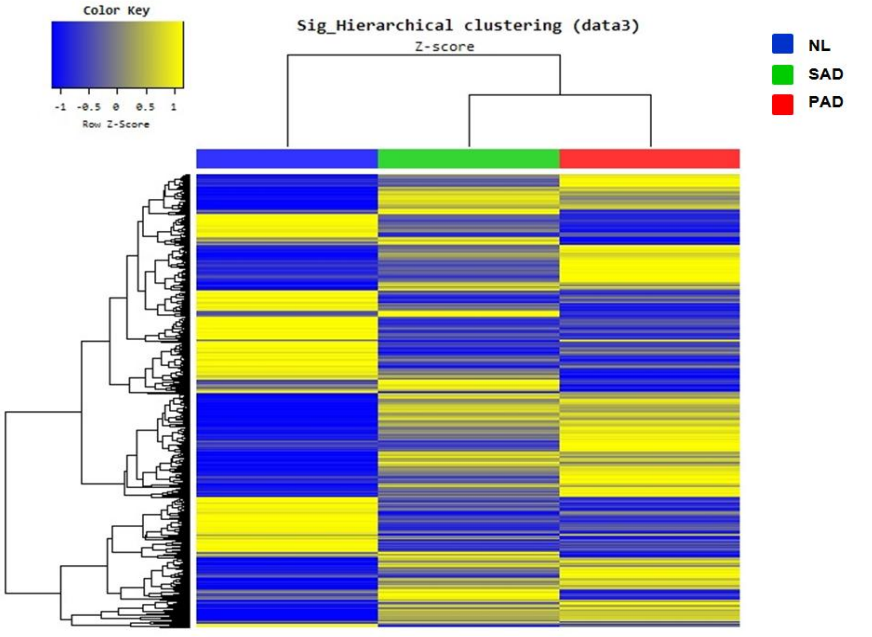


Figure 3. Overview of microarray data analysis using human NL, SAD and PAD skin. NL vs. SAD vs. PAD groups also exhibited distinct gene expression patterns, even though the NL group showed the most distinctive features with the opposite color key when compared with other groups.

Abbreviations: *NL*, normal; *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis

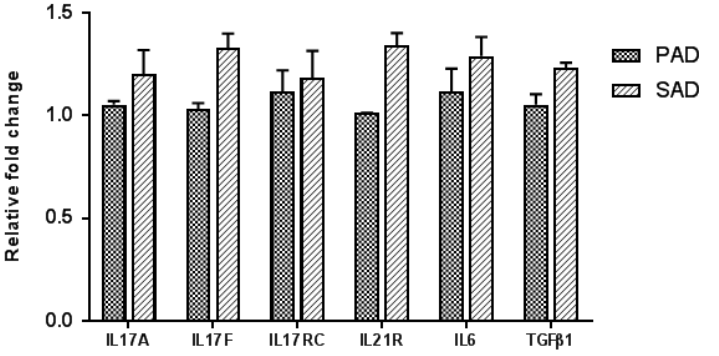


Figure 4. Microarray analysis of IL-17 and other cytokines related to IL-17 inflammation in human skin. Although statistical significances were not observed, IL-17 cytokine family, including IL-17A, IL-17F and its receptor IL-17RC showed fold increase. IL-21R, IL-6 and TGF- β1 that are related to IL-17 inflammation also exhibited fold increase in the SAD human skin.

Abbreviations: *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis

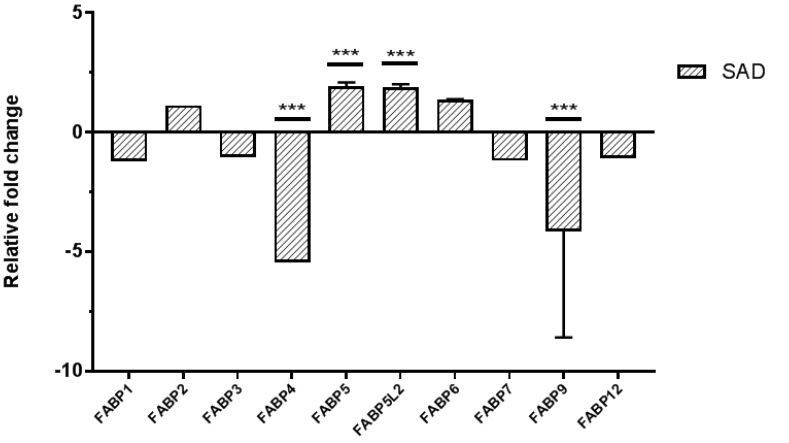


Figure 5. Relative fold changes of FABP family in SAD. According to the human skin microarray analysis, FABP5 and FABP5L2 showed significant fold increase, whereas FABP4 and FABP9 exhibited significant fold decrease. FABP2 and FABP6 revealed relatively increased fold change and FABP1, while FABP1, FABP3, FABP7 and FABP12 displayed relatively decreased fold change. ***p < 0.001.

Abbreviations: *FABP*, fatty acid binding protein; *SAD*, systemic atopic dermatitis

3. Confirmation of FABP4/5 expression in SAD mouse skin and visualization of FABP4/5 in human SAD skin

We performed qRT-PCR to see the gene expression of FABP4/5, as FABP4 and FABP5 share highly homologous sequence and structure with similar selectivity to fatty acids. As a result, higher expression of FABP5 was demonstrated in skin of SAD mouse, when compared to that of NL and PAD mouse models (Fig. 6). FABP4 was also increased in SAD mouse, but no statistical significance was observed when compared to the results of the NL and PAD mouse models.

In order to visualize the FABP expression in tissues, we used confocal microscopy to see FABP5 in human skin using immunofluorescence staining and confirmed a prominent FABP5 expression in SAD, while moderate FABP5 expression was seen in PAD skin, and minimal expression in NL skin (Fig. 7).

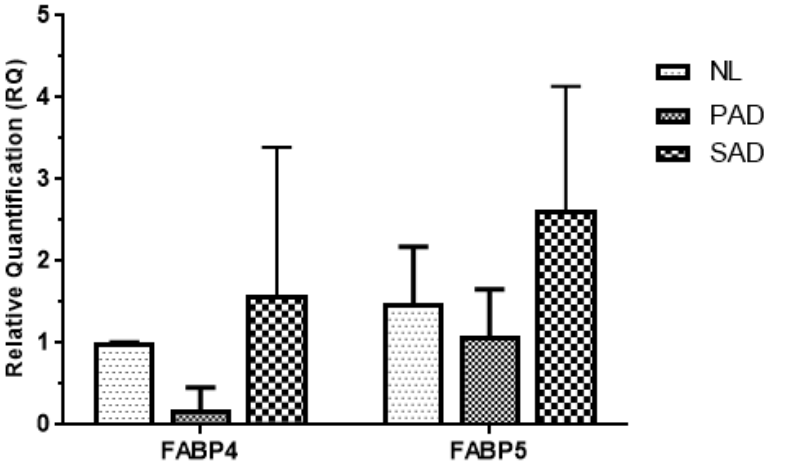


Figure 6. qRT-PCR result displayed increased FABP5 expression in the SAD mouse skin. As reported by qRT-PCR result to find the FABP4/5 expressions in skin of NL, PAD and SAD mouse models, FABP5 was elevated in SAD skin when compared with NL and PAD group. FABP4 was also highly expressed in SAD skin relatively, but no statistical significance was observed in both FABPs.
Abbreviations: *NL*, normal; *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis; *FABP*, fatty acid binding protein

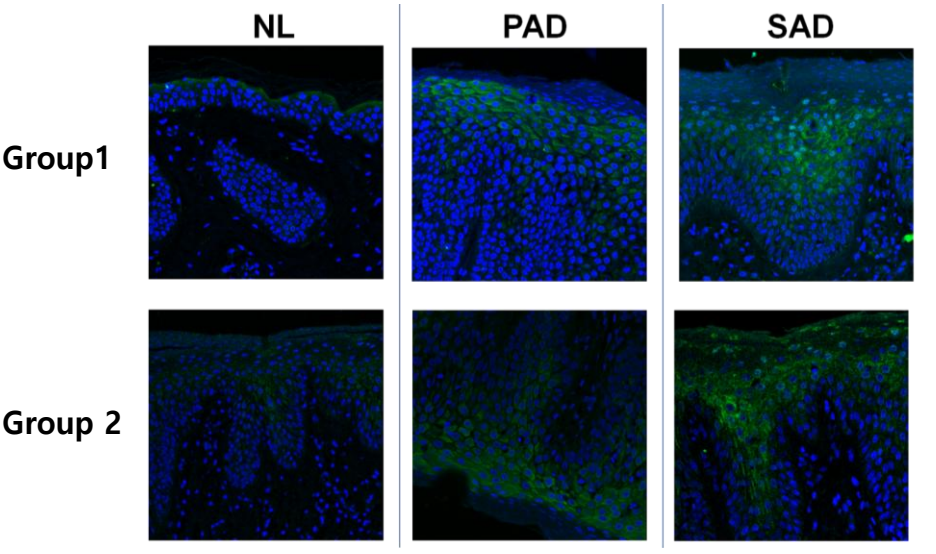


Figure 7. Immunofluorescence staining of FABP5 in NL, PAD and SAD human skin. Two skin samples from each group were collected and FABP5 was stained in green color. SAD skin samples revealed the most prominent FABP5 expression compared with NL and PAD samples. In PAD skin, moderate FABP5 expression was exhibited and NL skin displayed a minimal expression of FABP5. (x 200)

Abbreviations: *FABP*, fatty acid binding protein; *NL*, normal; *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis

4. Visualization of FABP4/5 in mouse SAD lung tissue

In order to investigate more evidences supporting the relationship between systemic inflammation and FABP expression, we obtained lung tissue from the OVA (ovalbumin) -induced asthma mouse model from a laboratory in Yonsei University College of Medicine, Institute of Allergy (Seoul, Korea) under Professor Jung-Won Park's supervision. Airway hyperresponsivenss was evaluated by methacholine challenge, according to increased concentrations of methacholine and the results were compared between NL and allergic asthma group. As a result, AA models showed significant airway hyperresponsivenss change, especially at 50 mg/ml of methacholine concentration in contrast to NL mouse models, clearly suggesting an exaggerated airway hyperresponsivenss in allergic asthma group (Fig. 8). Confocal microscopy with immunofluorescence staining was also used to visualize FABP expressions in lung tissues, which confirmed increased FABP4/5 expressions in the lung tissues of allergic asthma model (Fig. 9). FABP5 was mostly observed in respiratory bronchiole lining cells and FABP4 was stained in cellular portion around alveoli.

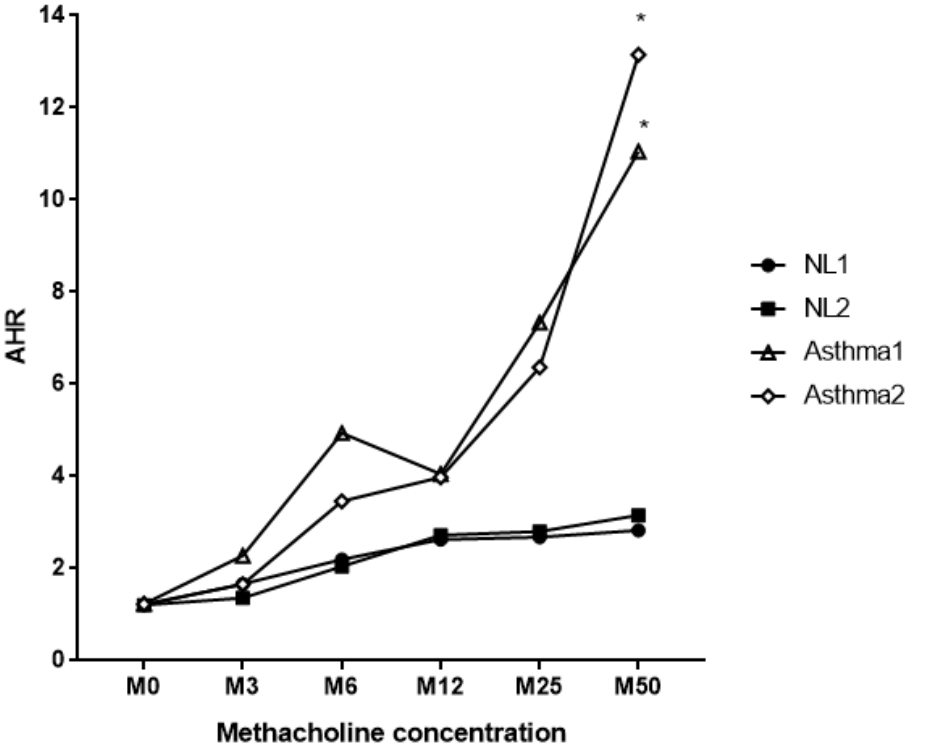


Figure 8. AHR examination by methacholine challenge after establishing OVA-induced asthma mouse model. AHR changes according to methacholine concentration were evaluated in both NL and allergic asthma groups. As a result, allergic asthma models showed a significant AHR change, especially at 50 mg/ml of methacholine concentration in contrast to NL mouse models. *$p < 0.05$
Abbreviations: *AHR*, airway hyperresponsiveness; *NL*, normal

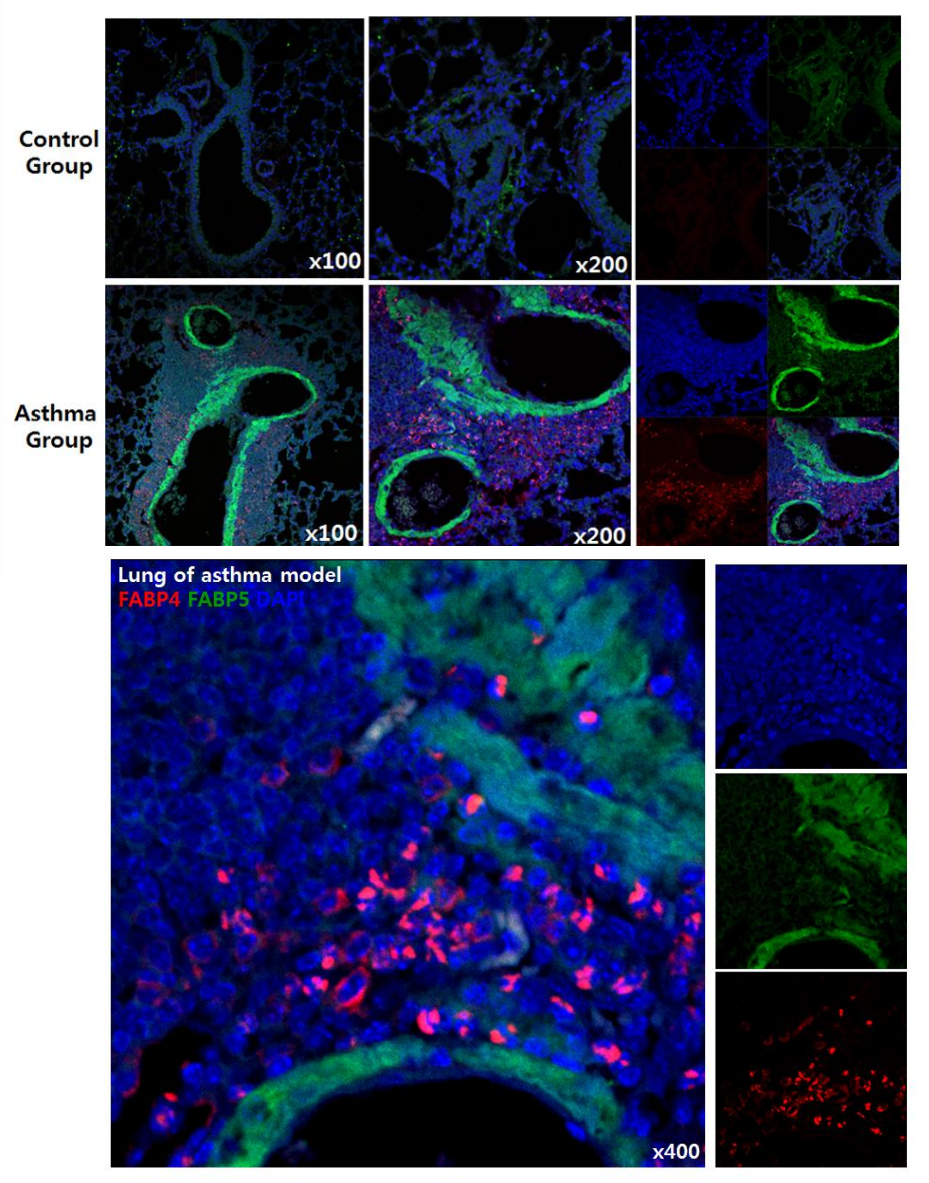


Figure 9. Increased FABP4 and FABP5 expression in lungs of OVA-induced asthma model. Immunofluorescence staining results were obtained by confocal microscopy (FABP4 in red and FABP5 in green, shown in x 100, x 200 and x 400). Note that FABP4 was mostly found in the cellular portion of lung tissue and FABP5 was observed in respiratory bronchiole lining cells.

Abbreviations: *FABP*, fatty acid binding protein; *OVA*, ovalumin

5. Demonstrations of CD4+ CD69+ FABP4/5+ positive T_{RM} (tissue-resident memory T) cells and CD4+ IL-17+ FABP4/5+ T cells in the human SAD skin by using triple immunofluorescence staining

Additional triple immunofluorescence staining using human NL, PAD and SAD skin confirmed that CD4+ CD69+ T_{RM} cells in the human SAD skin expressed apparently significant FABP4/5 than in both NL and PAD skin (Fig. 10). Also, CD4+ IL-17+ T cells showed the same results, confirming the possible role of FABP4/5+ T cells expressing IL-17 in SAD (Fig. 11).

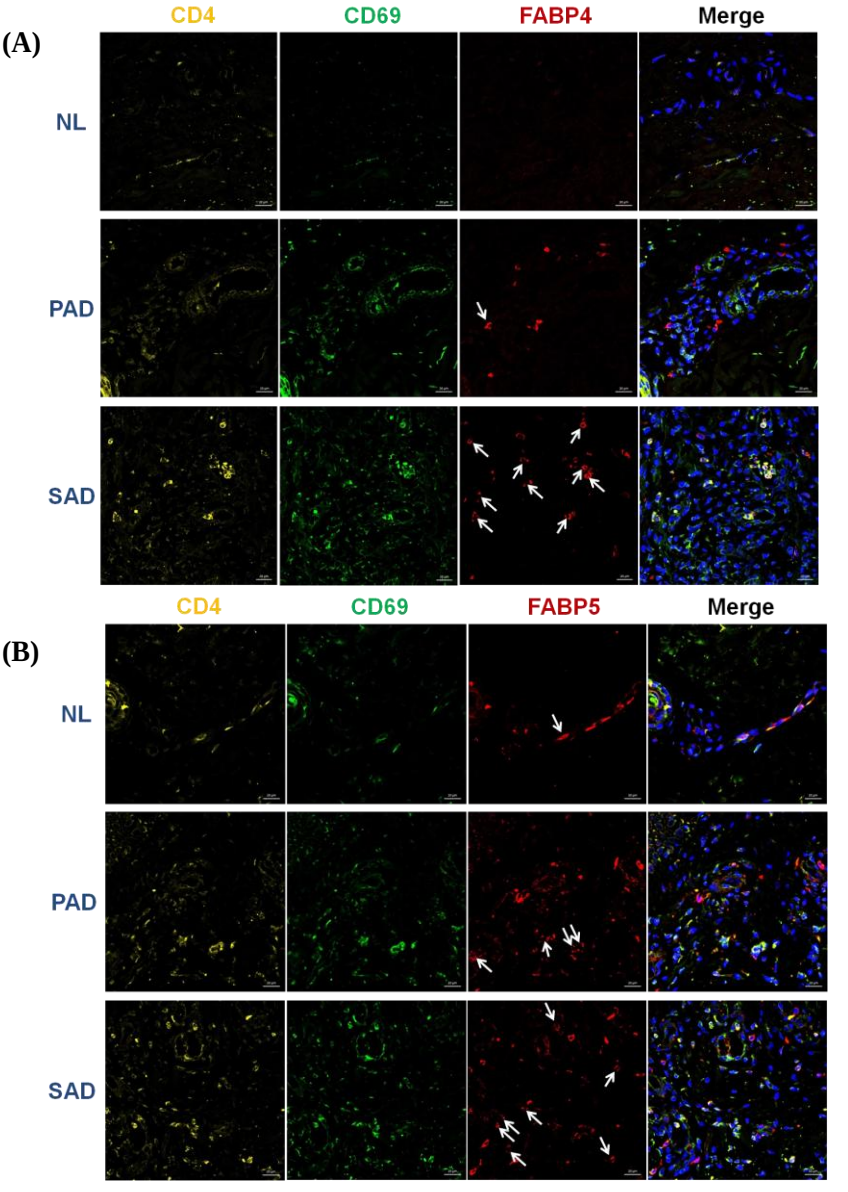


Figure 10. CD4+ CD69+ T_{RM} cells in the human SAD skin expressed significant FABP4 and FABP5. CD4+ CD69+ T_{RM} cells strongly expressing FABP4 (A) and FABP5 (B) were noted in SAD skin when compared with PAD and NL skin. Co-localization of CD4+ (yellow), CD69+ (green) T_{RM} cells with FABP4/5 (red) expression were indicated by white arrows. (x 400)

Abbreviations: *T_{RM}*, tissue-resident memory T; *FABP*, fatty acid binding protein; *SAD*, systemic atopic dermatitis; *PAD*, pure atopic dermatitis; *NL*, normal

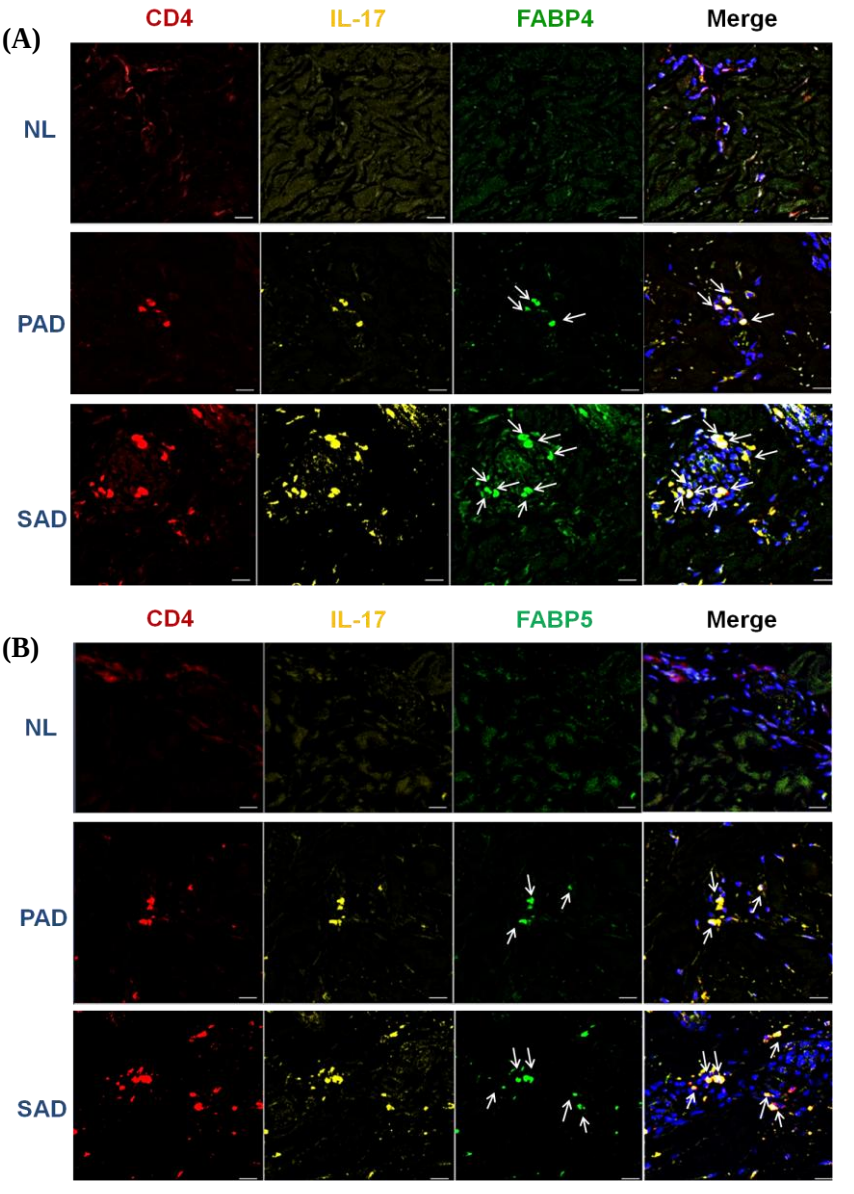


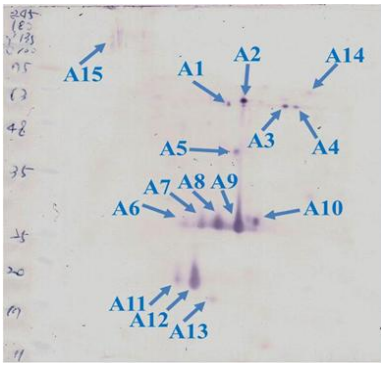
Figure 11. CD4+ IL-17+ T cells in the human SAD skin expressed significant FABP4 and FABP5. CD4+ IL-17+ T cells strongly expressing FABP4 and FABP5 were noted in SAD skin when compared with PAD and NL skin. Co-localization of CD4+ (red), IL-17+ (yellow) T cells with FABP4/5 (green) expression were indicated by white arrows. (x 400)

Abbreviations: *SAD, systemic atopic dermatitis; FABP,* fatty acid binding protein; *PAD,* pure atopic dermatitis; *NL,* normal

6. FABP5 expression after the treatment of Der f 1 in T cells

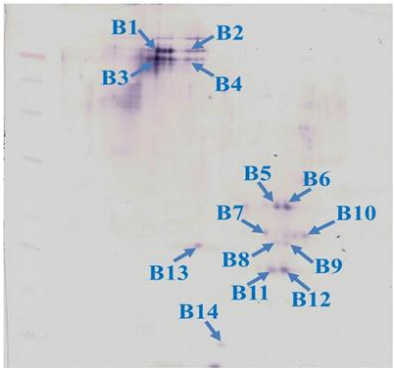
As mentioned previously, there was an apparent difference of *D. farinae* sensitization degree between SAD patients and PAD patients. And according to our previous study using 2D-western blot and ELISA, Der f 1 showed a specific relationship with SAD while Der f 14 displayed strong relationship with PAD (Fig. 12 to Fig. 14). So in the meantime, we carried out additional experiments to observe the expression of FABP5 induced by Der f 1 in SAD. The most significant FABP5 gene expression in T cells was confirmed in SAD by Der f 1 treatment (Fig. 15).

(A)



Spot	Identification (Organism)	Mass	Mascot score	NCBI Blast
A1	Nesprin-1 (*Cerapachys biroi*)	129,899	66	gi|607359598
A2	Not detected			
A3	Not detected			
A4	Nesprin-1 (*Cerapachys biroi*)	129,899	59	gi|607359598
A5	Not detected			
A6	Der f 1	23,763	209	gi|7413
A7	Der f 1	23,763	145	gi|7413
A8	Der f 1	23,763	208	gi|7413
A9	Der f 1	23,763	179	gi|7413
A10	Not detected			
A11	Ferritin	19,770	169	gi|442565878
A12	Ferritin	19,770	248	gi|442565878
A13	Ferritin	19,770	137	gi|442565878
A14	AGAP009853-PA-like protein (*Anopheles sinensis*)	46,460	58	gi|668458793
A15	Not detected			

(B)



Spot	Identification (Organism)	Mass	Mascot score	NCBI Blast
B1	Der p 14	190,542	97	gi|20385544
B2	Der f 11	102,407	510	gi|685432820
B3	Not detected			
B4	Der f 11	102,407	1055	gi|685432820
B5	Not detected			
B6	Der f 14	39,643	295	gi|729979
B7	PREDICTED: uncharaterized protein LOC101891104 (*Musca domestica*)	32,102	62	gi|557761217
B8	Not detected			
B9	Alpha-enolas	47,214	62	gi|605059430
B10	Not detected			
B11	Der f 14	39,643	65	gi|729979
B12	Der f 14	39,643	93	gi|729979
B13	Note detected			
B14	Not detected			

Figure 12. Images of 2D-Western blot to identify allergen fractions in SAD and PAD. (A) In SAD group, Der f 1 was the only detected allergen fraction among various HDM allergen fractions. (B) In PAD group, three allergen fractions, including Der p 14, Der f 11, Der f 14 were identified.
Abbreviations: *SAD*, systemic atopic dermatitis; *PAD*, pure atopic dermatitis; *HDM*, house dust mite

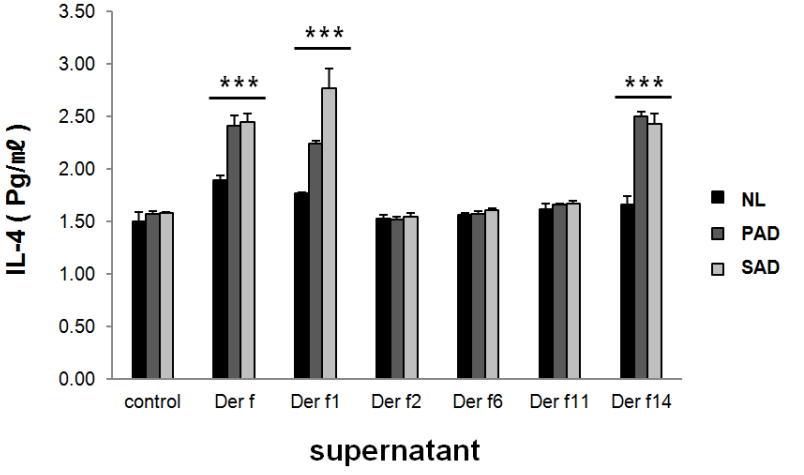


Figure 13. Result of ELISA for IL-4 after the co-culture of dendritic cells and CD4+ T cells. $CD4^+$ T cells, pre-activated with IL-2, were co-culture with $CD14^+$ PBMCs for 6 days at 37 ℃ and then 10μg/ml of each *D. farinae* fractions, including Der f 1, Der f 2, Der f 6, Der f 11, Der f 14 were treated and incubated for 48 hours at the same temperature. IL-4 ELISA was done by using the supernatant. Among various responses, Der f 1-treated T cells from SAD expressed strong immune responses. A similar response was shown by the whole *D. farinae* extract treatment. However, Der f 14 induced strongest immune responses in T cells from PAD. ***$p < 0.05$.

Abbreviations: *NL*, normal; *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis

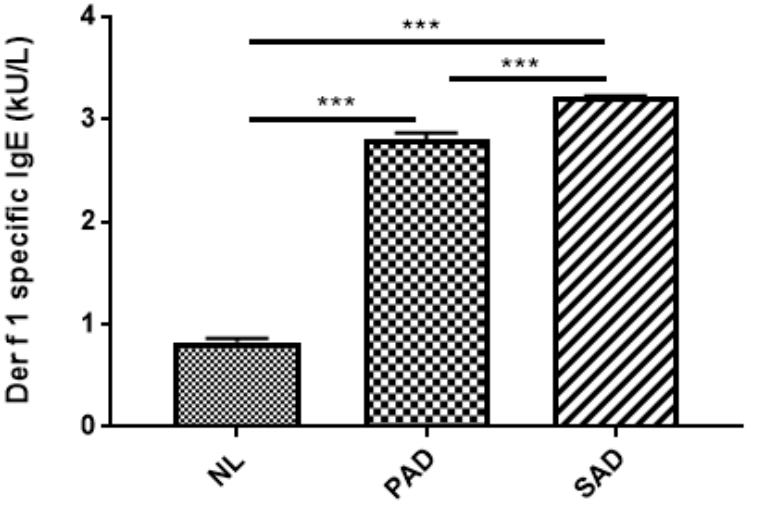


Figure 14. The ELISA result of Der f 1-specific IgE level of NL, PAD and SAD group using human blood samples. To confirm the specific IgE responses to Der f 1 in SAD, additional ELISA was done. Significant expression of Der f 1-specific IgE was observed in SAD when compared with both NL and PAD group. Also, the PAD group exhibited significant Der f 1-specific IgE in contrast to NL group. ***$p < 0.05$.

Abbreviations: *NL*, normal; *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis

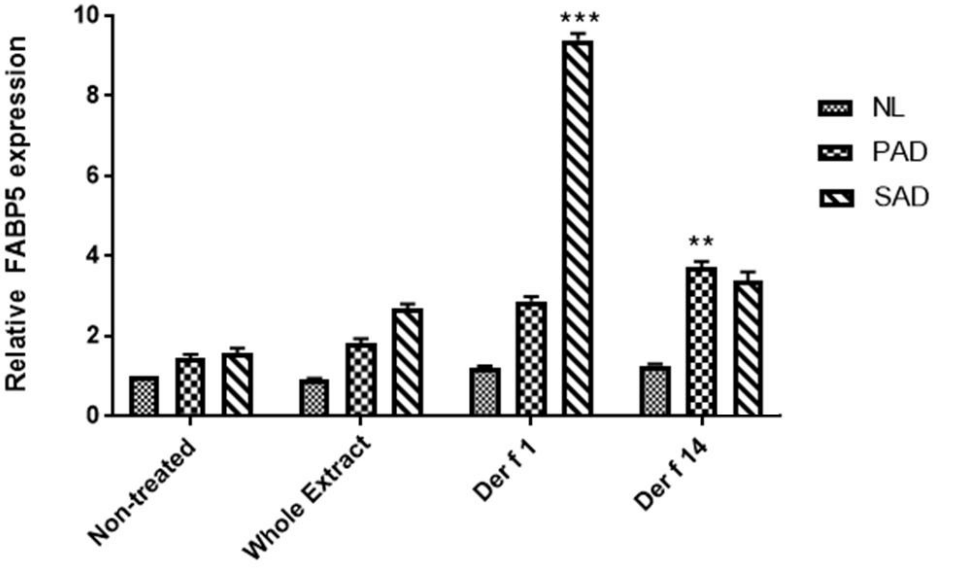


Figure 15. FABP5 expression in T cells under Der f 1, Der f 14 and whole *D. farinae* extract treatment. Dendritic cells and CD4+ T cells from human NL, PAD and SAD patients were co-cultured and *D. farinae* extracts including whole *D. farinae* extract, Der f 1, Der f 14 were treated and incubated for 48 hours for qRT-PCR. Der f 1 treated T cells from SAD expressed significant FABP5. In PAD group, Der f 14 treatments exhibited the most pronounced FABP5 expression. **p < 0.01, ***p < 0.001.

Abbreviations: *NL*, normal; *PAD*, pure atopic dermatitis; *SAD*, systemic atopic dermatitis; *FABP*, fatty acid binding protein

IV. DISCUSSION

While there are many supporting literatures verifying the idea of AD as a systemic disease, its pathomechanism is still unclear. Nonetheless, the possible role of IL-17 in SAD was previously reported.[34] In the experiment, IL-4/IL-13 double knockout mice were used and after epicutaneous sensitization with OVA, systemic immune responses to OVA including skin and airway inflammation, and AHR were measured. While Th2-driven responses were impaired and absent, intact dermal CD4+ cells were observed and an exaggerated IL-17 response mediated airway inflammation and AHR, which were reversed by IL-17 blockade. The study confirmed that epicutaneous sensitization in the absence of Th2 induced an intensified Th17 response systemically after antigen challenge resulting airway inflammation and AHR.[35]

As shown in our experiments, the SAD mouse model exhibited a significant IL-17 elevation both in the skin and other systemic organs, including lymph nodes and lung when compared with PAD and NL group. IL-4 representing Th2 cytokine was also increased in the SAD group, but it was less prominent than IL-17 elevation and statistical significance was only observed in skin and lymph nodes. These results were consistent with the allergic asthma mouse model experiment described in introduction part[11] and also shares common results with double knockout mice experiments which was mentioned above. Collectively, our result confirmed that not only Th2 but also Th17 immune responses take part in SAD.

RNA microarray analysis using human skin samples revealed similar results. There was a tendency toward fold increase of IL-17 family and Th17 pathway-related cytokines. By reviewing the approximately 100 genes with significant fold changes to find a candidate biomarker in SAD, we found a set of genes in relationship with fatty acid metabolism. Among these FABPs, FABP5 was chosen as a candidate protein.

FABP5 is also known as epidermal FABP (E-FABP) and psoriasis-associated FABP (PA-FABP).[36] FABP5 is detected in the granular layer of normal skin, where lipid synthesis is active so as to establish skin barriers. FABP5 is also

enhanced and expanded in psoriatic skin with hyperproliferation, impaired lipid metabolism and abnormal differentiation.[37] Besides, FABP5 null mice demonstrated barrier disruption and delayed wound healing due to impaired keratinocyte motility.[38]

FABP5 play roles in fatty acid uptake, transport and metabolism. Currently, FABP5 polymorphism only is known to be associated with type 2 diabetes mellitus. Nonetheless, there were several evidences supporting the relationship between FABP5 and AD. According to mass spectrometry study of AD skin, significant FABP5 expression was observed in acute and chronic AD lesion, minimal expression in non-lesional AD skin and no expression were founded by NL group.[39] Also, FABP5 showed correlation with local severity of AD involved skin, which implied FABP5 as a new horny layer marker protein for evaluating skin conditions in AD.[40]

In our study, FABP5 was selected as a candidate protein for several reasons in combination with the features of FABP5 that are described above. First, skin provides lipid-rich but nutrient-poor microenvironments and permeability barrier function of cornified layers requires lipid component which is acting as ‘mortar.’ in the skin barrier system. Also, lipid spillover is known to be a main initiator of IL-17 proinflammatory reaction in adipose tissue[41] which offers a possible relationship with Th17 pathway and fatty acid metabolism. According to current studies, the role of IL-17 is emphasized in the skin of psoriasis. It emerged as an IL-23/Th17-skewed disease and IL-17 promote keratinocytes proliferation and inflammatory responses. FABP5, which is a PA-FABP is highly expressed in psoriatic epidermis, suggesting the possible association with FABP5 and IL-17 immune responses.[42] Furthermore, according to the previous study which our group had participated, Asian AD phenotype demonstrated not only well-known Th2 predominance immune responses but also exhibited psoriatic phenotype with increased Th17 polarization, higher induction of Th17-related cytokines and IL-17-induced markers according to RT-PCR.[43] Extended experiments on SAD group were also performed before. One study analyzed sputum and nasal lavage from 4 groups: allergic rhinitis group, allergic rhinitis + allergic asthma group,

non- allergic rhinitis group and NL group. In allergic rhinitis + allergic asthma group, significant FABP5 expression was demonstrated and concluded that FABP5 might have contributed to airway remodeling and inflammation in allergic rhinitis + allergic asthma group.[44]

When we evaluated FABP5 expression by qRT-PCR, SAD mouse model skin revealed higher expression of FABP5 than PAD and NL skin. FABP5 immunofluorescence staining in human epidermis displayed none to minimal FABP5 expression in NL skin, moderate expression in PAD and prominent expression in SAD skin. To confirm the FABP5 expression in allergen-induced systemic inflammation, we used OVA-induced asthma mouse model. No FABP expression was shown in the control group and striking expressions of FABP4/5 were observed in asthma model.

The reason for FABP4/5 evaluation from the latter part of experiments is that current literatures had pointed out the similarities between those two genes; genes encoding FABP4 and FABP5 are closely related and they have highly homologous in sequence and structure, and bind to fatty acids with similar selectivity and affinity.[45] Furthermore, in a recent study to confirm FABP expression in T_{RM} cells using OVA-expressing vaccinia virus infected mouse model, FABP4/5 expression was significantly increased in CD8+ T_{RM} cells and FABP4/5 deficiency reduced long-term survival of T_{RM} cells in mouse skin, while having no effect on survival of central memory T cells.[46] These results were also observed in lung CD8+ T_{RM} cells with increased FABP4/5 expression after the intra-tracheal infection of OVA-expressing vaccinia virus, demonstrating the T-cell specific FABP4/5 expression after the airway inflammation. Enhanced extracellular free fatty acid uptake and increased FABP4/5 expression was conjointly shown in normal and psoriatic human CD8+ T_{RM} cells in the skin.

In triple immunofluorescence staining using human skin, no CD4+ CD69+ FABP4+ T_{RM} cells were shown in NL skin, only a few in PAD skin and significant cells were observed in SAD skin. The same result was demonstrated with CD4+ CD69+ FABP5+ T_{RM} cells. When we stained CD4+ IL-17+ FABP4+ T cells and CD4+ IL-17+ FABP5+ T cells, exactly same results were displayed, confirming

the relationship between SAD and FAPB5 expressing T cells showing Th17 differentiation. Since T_{RM} cells play roles in tissue-specific inflammatory disease,[47] skin inflammation might have induced Th17 response in this experiment.

The relationship between IL-17 and FABP5 had been previously reported. The literature explained that FABP5 expression in CD4+ T cells promoted Th17 cell differentiation.[48] IL-6 and TGF-β combination are well-known to up-regulate IL-17 production[49] and IL-6 is stimulated IL-21 induction and it sequentially induces retinoic acid-related orphan receptor (ROR) γt and RORα which in turn induces T cell expression of IL-17. However, impaired Th17 differentiation was demonstrated by FABP5-deficient CD4+ T cells under IL-6 stimulation, demonstrating the regulation of Th17 cell differentiation by FABP5.

Additional investigation using qRT-PCR in accordance with our preliminary study that *Der f 1* was the only identified allergen fraction of the SAD group was done to confirm the FABP5 expression in T cells under *Der f 1* treatment. The SAD group exhibited the most notable *Der f 1* specific IgE and FABP5 expression. However, in PAD group, *Der f 14* treatment induced the most prominent FABP5 expression, displaying consistent result with our preliminary study.

According to current knowledge, pathomechanism to explain AD in immune dysregulation aspects provide Th2-skewed immune responses resulting in down-regulation of epidermal barrier protein expressions, modulating IgE class switching, supporting Th2 cell survival and exacerbating pruritus, while Th22 cells and IL-22 inhibits terminal differentiation and induces epidermal hyperplasia.[50-52] Activated Th1 cells take a relatively partial role, especially in chronic AD. However, Th17 cells and IL-17 were thought to be less significant in AD.[50]

Nevertheless, based on our results, we assume that FABP5 expression in immature CD4+ T cells promote Th17 cell differentiation and IL-17 expression in SAD, whereas mainly Th2 cell differentiation and Th2-related cytokine expression takes place in PAD. All things considered, we illustrated a possible role of FABP5+ Th17 immune responses in the development of SAD (Fig. 16).

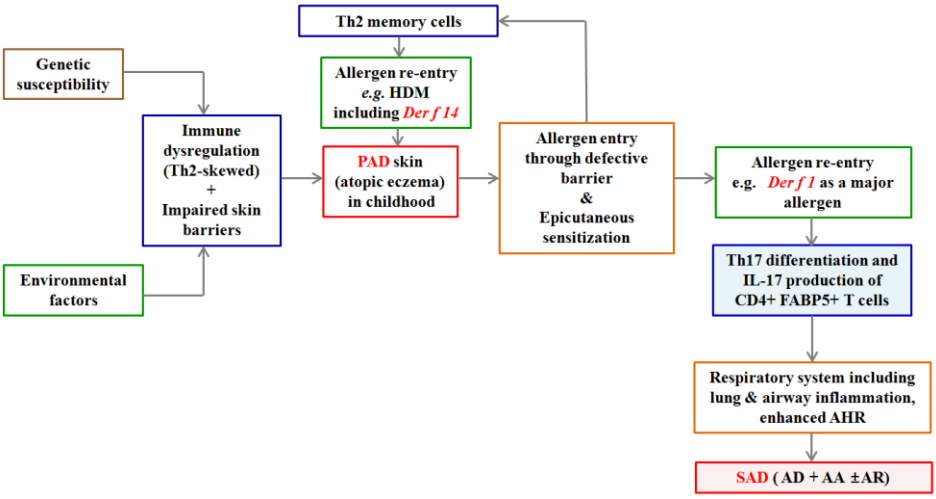


Figure 16. Possible role of Th17 immune responses of CD4+ FABP5+ T cells in the development of SAD. Genetic susceptibility, including mutations in barrier proteins (e.g. filaggrin mutation) and environmental factors such as sensitization to allergens and infection induce Th2-skewed immune dysregulation and impaired skin barriers. Skin inflammation and eczematous lesion develop, and additional allergen entry through defective skin barrier and epicutaneous sensitization takes a place. Th2 memory cells are produced and such as *Der f 14* allergen fraction re-entry exacerbates and maintains PAD. However, in SAD, *Der f 1* re-entry through impaired barrier promotes Th17 differentiation and IL-17 production of CD4+ FABP5+ T cells, which in turn induces lung and airway inflammation, eventually leading to SAD development.

Abbreviations: *SAD*, systemic atopic dermatitis; *AD*, atopic dermatitis; *AA*, allergic asthma; *AR*, allergic rhinitis; *PAD*, pure atopic dermatitis; *FABP*, fatty acid binding protein

CONCLUSION

Taken together, IL-17-mediated cutaneous inflammation of CD4+ FABP5+ T cells in SAD may explain a possible pathomechanism of atopic dermatitis as a systemic disease. Further study will be necessary for evaluating the role of FABP5 in SAD.

REFERENCES

1. Boguniewicz M, Leung DY. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. Immunol Rev. 2011;242:233-46.
2. Bawazeer AM, Hodge WG, Lorimer B. Atopy and keratoconus: a multivariate analysis. Br J Ophthalmol. 2000;84:834-6.
3. Bair B, Dodd J, Heidelberg K, Krach K. Cataracts in atopic dermatitis: a case presentation and review of the literature. Arch Dermatol. 2011;147:585-8.
4. Tzanakis NE, Tsiligianni IG, Siafakas NM. Pulmonary involvement and allergic disorders in inflammatory bowel disease. World J Gastroenterol. 2010;16:299-305.
5. Niwa Y, Sumi H, Akamatsu H. An association between ulcerative colitis and atopic dermatitis, diseases of impaired superficial barriers. J Invest Dermatol. 2004;123:999-1000.
6. Salsano ME, Graziano L, Luongo I, Pilla P, Giordano M, Lama G. Atopy in childhood idiopathic nephrotic syndrome. Acta Paediatr. 2007;96:561-6.
7. Yap HK, Yip WC, Lee BW, Ho TF, Teo J, Aw SE, et al. The incidence of atopy in steroid responsive nephrotic syndrome: clinical and immunological parameters. Ann Allergy. 1983;51:590-4.
8. Kokkonen J, Niinimaki A. Increased incidence of autoimmune disorders as a late complication in children with early onset dermatitis and/or milk allergy. J Autoimmun. 2004;22:341-4.
9. Pedulla M, Miraglia Del Giudice M, Fierro V, Arrigo T, Gitto E, et al. Atopy as a risk factor for thyroid autoimmunity in children. J Biol Regul Homeost Agents. 2012;26:S9-S14.
10. Rabin RL, Levinson AI. The nexus between atopic disease and autoimmunity: a review of the epidemiological and mechanistic literature. Clin Exp Immunol. 2008;153:19-30.
11. Mittermann I, Aichberger KJ, Bunder R, Mothes N, Renz H, Valenta R. Autoimmunity and atopic dermatitis. Curr Opin Allergy Clin Immunol. 2004;4:367-71.
12. Darlenski R, Kazandjieva J, Hristakieva E, Fluhr JW. Atopic dermatitis as a systemic disease. Clin Dermatol. 2014;32:409-13.

13. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol. 2004;113:832-6.
14. Plotz SG, Ring J. What's new in atopic eczema? Expert Opin Emerg Drugs. 2010;15:249-67.
15. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. J Allergy Clin Immunol. 2003;112:S118-27.
16. Spergel JM. Atopic march: link to upper airways. Curr Opin Allergy Clin Immunol. 2005;5:17-21.
17. Bantz SK, Zhu Z, Zheng T. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. J Clin Cell Immunol. 2014;5:202.
18. Guilbert TW, Morgan WJ, Zeiger RS, Bacharier LB, Boehmer SJ, Krawiec M, et al. Atopic characteristics of children with recurrent wheezing at high risk for the development of childhood asthma. J Allergy Clin Immunol. 2004;114:1282-7.
19. Kapoor R, Menon C, Hoffstad O, Bilker W, Leclerc P, margolis DJ. The prevalence of atopic triad in children with physician-confirmed atopic dermatitis. J Am Acad Dermatol. 2008;58:68-73.
20. Kulig M, Bergmann R, Klettke U, Wahn V, Tacke U, Wahn U. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. J Allergy Clin Immunol. 1999;103:1173-9.
21. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med. 1995;332:133-8.
22. Ricci G, Patrizi A, Baldi E, Menna G, Tabanelli M, Masi M. Long-term follow-up of atopic dermatitis: retrospective analysis of related risk factors and association with concomitant allergic diseases. J Am Acad Dermatol. 2006;55:765-71.
23. van der Hulst AE, Klip H, Brand PL. Risk of developing asthma in young children with atopic eczema: a systematic review. J Allergy Clin Immunol. 2007;120:565-9.

24. Hogan MB, Peele K, Wilson NW. Skin barrier function and its importance at the start of the atopic march. J Allergy (Cairo) 2012;2012:901940.
25. Woodward AL, Spergel JM, Alenius H, Mizoguchi E, Bhan AK, Castigli E, et al. An obligate role for T-cell receptor alphabeta+ T cells but not T-cell receptor gammadelta+ T cells, B cells, or CD40/CD40L interactions in a mouse model of atopic dermatitis. J Allergy Clin Immunol 2001;107:359-66.
26. Herrick CA, MacLeod H, Glusac E, Tigelaar RE, Bottomly K. Th2 responses induced by epicutaneous or inhalational protein exposure are differentially dependent on IL-4. J Clin Invest 2000;105:765-75.
27. Herrick CA, Bottomly K. To respond or not to respond: T cells in allergic asthma. Nat Rev Immunol 2003;3:405-12.
28. Leyva-Castillo JM, Hener P, Jiang H, Li M. TSLP produced by keratinocytes promotes allergen sensitization through skin and thereby triggers atopic march in mice. J Invest Dermatol 2013;133:154-63.
29. Dharmage SC, Lowe AJ, Matheson MC, Burgess JA, Allen KJ, Abramson MJ. Atopic dermatitis and the atopic march revisited. Allergy 2014;69:17-27.
30. Pacciani V, Corrente S, Gregori S, Pierantozzi A, Silenzi R, Chianca M, et al. Correlation of Der p 2 T-cell responses with clinical characteristics of children allergic to house dust mite. Ann Allergy Asthma Immunol. 2012;109:442-7.
31. Ullah MA, Revez JA, Loh Z, Simpson J, Zhang V, Bain L, et al. Allergen-induced IL-6 trans-signaling activates γδ T cells to promote type 2 and type 17 airway inflammation. J Allergy Clin Immunol. 2015;134:1065-73.
32. Bin L, Leung DY. Genetic and epigenetic studies of atopic dermatitis. Allergy Asthma Clin Immunol. 2016;12:52. eCollection 2016.
33. Weyand CM, Goronzy JJ. Immune mechanisms in medium and large-vessel vasculitis. Nat Rev Rheumatol. 2013;9:731-40.
34. Lee H, Shin JU, Lee J, Chu H, Lee KH. Characteristics of Atopic Dermatitis in a Post-childhood Atopic March Group. Korean J Dermatol. 2017;55:110-5.

35. He R, Kim HY, Yoon J, Oyoshi MK, MacGinnitie A, Goya S, et al. Exaggerated IL-17 response to epicutaneous sensitization mediates airway inflammation in the absence of IL-4 and IL-13. J Allergy Clin Immunol. 2009;124:761-70.
36. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. Nat Rev Drug Discov. 2008;7:489-503.
37. Siegenthaler G, Hotz R, Chatellard-Gruaz D, Didierjean L, Hellman U, Saurat JH. Purification and characterization of the human epidermal fatty acid-binding protein: localization during epidermal cell differentiation in vivo and in vitro. Biochem J. 1994;302:363-71.
38. Owada Y, Takano H, Yamanaka H, Kobayashi H, Sugitani Y, Tomioka Y, et al. Altered water barrier function in epidermal-type fatty acid binding protein-deficient mice. J Invest Dermatol. 2002;118:430-5.
39. Broccardo CJ, Mahaffey SB, Strand M, Reisdorph NA, Leung DY. Peeling off the layers: skin taping and a novel proteomics approach to study atopic dermatitis.J Allergy Clin Immunol. 2009;124:1113-5.
40. Yamane Y, Moriyama K, Yasuda C, Miyata S, Aihara M, Ikezawa Z, et al. New horny layer marker proteins for evaluating skin condition in atopic dermatitis. Int Arch Allergy Immunol. 2009;150:89-101.
41. Mraz M, Haluzik M. The role of adipose tissue immune cells in obesity and low-grade inflammation.J Endocrinol. 2014;222:R113-27.
42. Dallaglio K, Marconi A, Truzzi F, Lotti R, Palazzo E, Petrachi T, et al. E-FABP induces differentiation in normal human keratinocytes and modulates the differentiation process in psoriatic keratinocytes in vitro. Exp Dermatol. 2013;22:255-61.
43. Noda S, Suárez-Fariñas M, Ungar B, Kim SJ, de Guzman Strong C, Xu H, et al. The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. J Allergy Clin Immunol. 2015;136:1254-64.
44. Suojalehto H, Kinaret P, Kilpeläinen M, Toskala E, Ahonen N, Wolff H, et al. Level of fatty acid binding protein 5 (FABP5) is increased in sputum of allergic asthmatics and links to airway remodeling and inflammation. PLoS One. 2015;10:e0127003.

45. Furuhashi M, Saitoh S, Shimamoto K, Miura T. Fatty Acid-Binding Protein 4 (FABP4): Pathophysiological insights and potent clinical biomarker of metabolic and cardiovascular diseases. Clin Med Insights Cardiol. 2015;8:23-33.
46. Pan Y, Tian T, Park CO, Lofftus SY, Mei S, Liu X, et al. Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. Nature. 2017;543:252-6.
47. Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. Nat Med. 2015;21:688-97.
48. Li B, Reynolds JM, Stout RD, Bernlohr DA, Suttles J. Regulation of Th17 differentiation by epidermal fatty acid-binding protein. J Immunol. 2009;182:7625-33.
49. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol. 2007;8:1390-7.
50. Guttman-Yassky E, Dhingra N, Leung DY. New era of biologic therapeutics in atopic dermatitis. Expert Opin Biol Ther. 2013;13:549-61.
51. Wolf R, Wolf D. Abnormal epidermal barrier in the pathogenesis of atopic dermatitis. Clin Dermatol. 2012;30:329-34.
52. Gittler JK, Shemer A, Suárez-Fariñas M, Fuentes-Duculan J, Gulewicz KJ, Wang CQ, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. J Allergy Clin Immunol. 2012;130:1344-54.

ABSTRACT (IN KOREAN)

전신질환을 동반한 아토피피부염에서 fatty acid binding protein 5를 발현하는 CD4+ T 세포가 생성하는 interleukin-17의 역할

<지도교수 이 광 훈>

연세대학교 대학원 의학과

이 정 수

단순한 피부질환이 아닌, 전신 질환으로서의 아토피피부염을 설명하는 근거들이 증가하는 가운데 가장 명확한 증거로 '아토피 행진'을 꼽을 수 있다. 이는 시간이 흐름에 따라 영유아기 피부 습진으로 시작하여 유소아기의 천식, 비염등의 호흡기 알레르기 증상을 보이는 순차적 이행을 보이는 현상을 의미한다. 그러나 현재까지 전신질환으로서의 아토피피부염의 기전에 대해 명확이 밝혀진 바가 없어 이를 규명하기 위해 전신 아토피피부염의 면역학적 특징과 생물학적 표지자를 찾기 위한 연구를 진행하였다.

먼저 NC/Nga 쥐를 이용하여, 집먼지 진드기의 반복적인 피부 감작을 통해 단순아토피피부염 모델을 유발하였으며, 추가적인 호흡기 감작을 이용한 전신아토피피부염 모델을 유발하였다. 이후 정상 대조군, 단순아토피피부염, 전신아토피피부염 쥐 모델 및 인간의 조직과 T 세포를 이용한 RNA 유전자미세배열(microarray) 분석법, 정량적 실시간 중합효소 연쇄반응 (qRT-PCR) 및 면역형광 염색을 시행하였다.

다양한 실험을 통해 단순아토피피부염과 전신아토피피부염 사이에 의미있는 면역학적 특징의 차이를 관찰할 수 있었는데, 먼저 전신아토피피부염 쥐 모델의 피부 및 다기관 조직에서 IL-17의 증가 소견이 뚜렷하게 나타났다. 또한 인간 피부를

이용하여 진행한 RNA microarray 분석을 통하여 IL-17 및 관련된 염증 사이토카인의 발현 증가를 확인할 수 있었다. IL-17은 특히 전신적인 염증반응과 밀접한 관계가 있으며 피부를 포함한 지질이 풍부한 환경에서의 염증반응에 관여하는 사이토카인이므로, microarray 분석을 통해 2배 이상의 매우 의미있는 증가 혹은 감소를 보이는 유전자들 중, 지질의 대사와 관련이 있는 지방산결합단백 5를 동정하였다. 또한 qRT-PCR 및 면역형광염색을 통해 전신아토피피부염 쥐 모델 및 인간 피부에서 지방산결합단백 5가 모두 증가되어있다는 사실을 확인하였다. 마지막으로 단순아토피피부염환자에 비해 전신아토피피부염환자의 피부에 있는 CD4+ CD69+ T_{RM} 세포와 CD4+ IL-17+ T 세포에서 지방산결합단백 4/5 발현이 모두 증가되어 있음을 관찰하였다.

이를 종합해볼 때 지방산결합단백 5를 발현하는 CD4+ T cell의 IL-17 생산 및 이의 매개를 통한 전신아토피피부염의 발생 기전을 유추해 볼 수 있다.

핵심되는 말: 전신아토피피부염, 지방산결합단백 5, IL-17, T_{RM} 세포