Identification of the dendritic cell subset critical for the type 2 immune response in murine contact hypersensitivity model

Jae Won Lee

Department of Medicine

The Graduate School, Yonsei University

Identification of the dendritic cell subset critical for the type 2 immune response in murine contact hypersensitivity model

Directed by Professor Min-Geol Lee

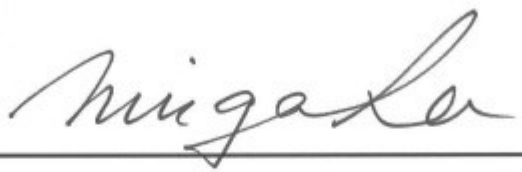
The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

Jae Won Lee

December 2017

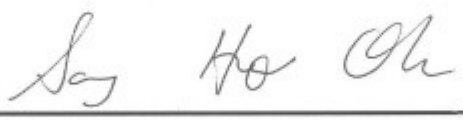
연세대학교
YONSEI UNIVERSITY

This certifies that the Master's Thesis of Jae Won Lee is approved.



Thesis Supervisor: Min-Geol Lee

Thesis Committee Chair: Chae Gyu Park



Thesis Committee Member: Sang Ho Oh

The Graduate School
Yonsei University

December 2017

ACKNOWLEDGEMENTS

The final outcome of this study required much guidance and inspiration from Professor Min-Geol Lee. I would like to deeply thank him for all his support and encouragement along the completion of this study.

I also owe my profound gratitude to Professor Chae Gyu Park and Professor Sang Ho Oh who took keen interest on this study and offered invaluable professional advices and guidance.

I thank Tae-Gyun Kim for his priceless advices, and Jeyun Park and Sung Hee Kim for their kind support and assisstances. I also thank my colleagues in the lab and clinic. Last but not least, I thank my family for their unchanging presence in my life.

<TABLE OF CONTENTS>

LIST OF FIGURES

ABSTRACT

Identification of the dendritic cell subset critical for the type 2 immune response in murine contact hypersensitivity model

Jae Won Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Min-Geol Lee)

Allergic contact dermatitis is one of the most common skin diseases, affecting 15-20% of the general population worldwide. Murine contact hypersensitivity (CHS) is one of the most frequently used animal models of allergic contact dermatitis. As new subsets of dermal dendritic cells (DCs) with specific surface markers and functions are being identified, the exact role of each DC subset in CHS remains unanswered. Specifically, DC subsets responsible for the type 2 immune response in the murine CHS model have not been investigated previously. The aim of this study is to validate the increase of type 2 immune response and to explore the functional role of DC subsets in acute CHS mouse model induced by oxazolone (OXA).

In the present study, 50μl of 3% OXA was applied on the shaved abdominal skin of mice, and the sensitized mice were challenged by applying 10μl of 0.6% OXA to the left ear and 10μl of vehicle on the right. During the sensitization phase, mRNA levels of cytokines were analyzed in skin draining lymph nodes (SDLNs) sequentially after single epicutaneous sensitization with OXA. Then I compared the ear thickness and mRNA levels of cytokines at 24 hrs after challenge with OXA in wild type mice and diphtheria toxin (DT)-treated Mgl2-DTR mice to examine functional role of $CD301b^+$ dermal DCs. Similar processes were done to Langerin-DTR mice to determine whether Langerhans cells and/or $Langerin^+$ dermal DCs has a critical role.

In the sensitization phase, the levels of IL-4 transcript in SDLNs of wild type mice were markedly increased starting from the 72hr time point compared to the baseline, while those of IFN-γ did not show significant increase. During the challenge phase, the extent of increase in both ear thickness and mRNA level of IL-4 were significantly lower in the DT-treated Mgl2-DTR mice compared to the wild type control mice. The levels of IFN-γ did not show significant differences in the absence of $CD301b^+$ dermal DCs. On the other hand, OXA-induced acute CHS responses as well as cytokine expression patterns of the DT-treated Langerin-DTR mice were comparable to the wild type control mice.

The findings of this study could suggest that in OXA-induced CHS model, type 2 immunity seems to be crucial for the cutaneous inflammation, and $CD301b^+$ dermal DC subset is responsible for mediating this immune reaction.

Key words : Allergic contact dermatitis, Contact hypersensitivity, Dendritic cells, Mgl2-DTR mouse, Langerin-DTR mouse, Interleukin-4, Cutaneous immunology

Identification of the dendritic cell subset critical for the type 2 immune response in murine contact hypersensitivity model

Jae Won Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Min-Geol Lee)

I. INTRODUCTION

Allergic contact dermatitis (ACD) is one of the most common skin diseases, affecting 15-20% of the general population worldwide.[1] It is a cutaneous immune reaction against one or more nontoxic allergens that come in contact with the skin. It is characterized by intensely pruritic erythematous patches, edema and occasional vesicles. It is induced by allergens or haptens, which are small chemically reactive molecules. Detection and avoidance of allergens are very difficult and medical treatment with either systemic or topical immunosuppressive agents including corticosteroids is often necessary. Therefore, ACD can affect patients for years and is a grave socioeconomic problem.[2]

ACD is classified as a delayed-type hypersensitivity response to antigens that come in contact with the skin. Two distinct phases are involved in ACD: sensitization phase and elicitation phase. The sensitization phase represents the first contact with the allergen and has no clinical manifestations, and the elicitation phase occurs on re-challenge, producing visible dermatitis. Murine contact hypersensitivity (CHS) is one of the most frequently used animal models of ACD. It was previously believed that CHS was mainly mediated by Th1 differentiation and regulated by Th2 cells.[3] However, as each hapten may

have its own properties to induce a dominant type of helper T cell subset, this factor needs to be considered when interpreting the role of each T cell subset in CHS model.[4,5]

Among haptens used in CHS, oxazolone (OXA), trinitrochlorobenzene (TNCB), 1-chloro-2,4-dinitrobenzene (DNCB), and 1-fluoro-2,4-dinitrobenzne (DNFB) have generally been regarded as Th1-inducing haptens, whereas fluorescein isothiocyanate is regarded as inducing Th2-inducing hapten.[6] A previous report demonstrated that repeated elicitation with hapten results in a shift from Th1- to Th2-mediated cutaneous inflammation, which mimics atopic dermatitis.[7] Accordingly, in previous laboratory studies which needed Th2-mediated inflammation model, OXA has been used for the setting of chronic CHS models in which repetitive hapten-challenging is needed. However, I unexpectedly found the evidence of increased Th2 differentiation in the acute stage of CHS in the process of another study regarding chronic hypersensitivity (Unpublished observation). In addition, another study by Tkalcevic *et al*. presented the increased protein level of both IL-4 and IFN-γ in ear tissues in acute phase of OXA-induced CHS murine models.[8] These conflicted results of previous studies facilitate the needs of further investigation regarding the exact pattern of T cell response induced in acute phase of OXA-induced CHS murine model.

Dendritic cell (DC) is called a 'bridging cell' connecting the innate and acquired immunity in that it has potent antigen-presenting ability to promote antigen-specific T-cell response and antibody production.[9] DCs have been revealed to have a crucial role in CHS.[10-12] DCs of the murine skin can be largely categorized into Langerhans cells (LCs), Langerin$^+$ DCs, and Langerin$^-$ DCs. LCs reside in the epidermis and they are characterized by the expression of Langerin/CD207, although other Langerin-expressing dermal DCs population has been reported recently. Dermal DCs (dDCs) consist of two main subsets, namely Langerin$^+$ DCs, and Langerin$^-$ DCs, and the majority of dDCs

are Langerin negative.[13] Although LCs were conventionally considered to be the main antigen presenting cells in the sensitization phase of CHS, recent studies using new depletion techniques have provided controversial results showing that the depletion of LCs resulting in promotion and suppression of CHS as well as no effect on CHS. Furthermore, Langerin$^+$ dDCs were reported to be involved in the initiation of CHS responses, and another recent study showed that antigen presentation by Langerin$^+$ dDCs alone did not appear to represent the main pathway in sensitization phase. CD301b$^+$ dDC is a unique subset of dDCs expressing macrophage galactose-type C-type lectin 2 (Mgl2/CD301b) which was recently proven to be expressed on a portion of conventional dDCs that were sufficient to initiate CHS in vivo. CD301b$^+$ DC is characterized by a lack of expressing Langerin, EpCAM, and CD103 markers and requires Irf4 transcription factor for its development, indicating that it might belong to the dermal Langerin$^-$ lineage of the skin.[14] Functional studies have revealed that CD301b$^+$ dDCs were involved in inducing Th2 immune response, regulating IL-17A production from dermal γδT cells, and limiting follicular helper T cell response.[14,15] Still, the precise immunological role of CD301b$^+$ DCs in the skin has not been fully understood. As new subsets of DCs with specific surface markers and functions are being identified, the exact role of each DC subset in acute and chronic CHS remains controversial.[16-18] Specifically, DC subsets responsible for the Th2 immune reaction in the setting of acute CHS model induced by OXA have not been investigated previously.

The aim of this study is to investigate the exact pattern of T cell differentiation in acute phase of OXA-induced CHS murine model as well as to identify the DC subsets which have major role in this specific immune response.

II. MATERIALS AND METHODS

1. Mice

Mice carrying transgene of Mgl2 promoter-driven DTR (Mgl2-DTR mice) were kindly provided by Akiko Iwasaki at Yale University. Mice carrying Langerin promoter-driven DTR (Langerin-DTR mice) were a gift from Heung Kyu Lee at Korea Advanced Institute of Science and Technology. Age- and sex-matched littermate mice devoid of transgene were used as wild-type (WT) controls (C57BL/6 mice) throughout the study. Six- to 12-week-old mice that were bred in specific pathogen-free facilities at Yonsei University College of Medicine were used for all experiments. All animal studies were approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine.

2. Sensitization and elicitation of contact hypersensitivity

CHS to OXA (Sigma Aldrich, St. Louis, MO, USA) was induced by sensitization on day 0 by exposing mice to 50μl of 3% OXA in acetone/olive oil (4:1) on their shaved abdominal skin. To evaluate the immune response during sensitization phase, skin draining lymph nodes (SDLNs) of axilla were extracted at day 0, day 1, day 3, and day 5 before the OXA-challenge. From those tissue, mRNA levels of cytokines and chemokines were analyzed by RT-PCR. At day 5, the sensitized mice were challenged by applying 10μl of 0.6% OXA to the left ear and 10μl of vehicle on the right ear. 24 hours later, ear thickness was measured using a caliper. The net response in ear swelling was calculated as follows: thickness of ears after hapten challenge - thickness of ears before hapten application. Using RT-PCR, mRNA expressions of cytokines and chemokines in excised ear tissues were analyzed.

3. Tissue preparation and flow cytometry

To induce the depletion of specific DC subsets, 1μg diphtheria toxin (DT, Sigma) was injected intraperitoneally to Mgl2-DTR and Langerin-DTR mice 24hr before sacrifice. Ear tissues were incubated for 30min at 37℃ in 2mg/ml Dispase-II (Roche Diagnositcs GmbH, Mannheim, Germany). Epidermal and dermal sheets were digested in 1mg/ml collagenase IV (Worthington, Lakewood, CA, USA) and 0.1mg/ml DNase I (Roche) for 1.5hr at 37℃ and passed through 70μm strainer to generate single cell suspension. Lymph nodes were teased using forceps and similarly processed as ear tissues. Harvested cells were stained for 30min at 4℃ in PBS containing 1% FBS with following anti-mouse antibodies: Fc blocker, CD45, EpCAM, CD64, CD24, CD11b, CD301b, MHC II, and CD11c (Biolegend, San Diego, CA, USA**).** Samples were measured on a FACS Forttessa (BD Biosciences, San Jose, CA, USA) and analyzed using Flow Jo software (Treestar, Ashland, OR, USA).

4. Histopathology

Excised ear sections were prepared with Cryostat (Leica, Wetzlar, Germany). Tissue sections were stained with hematoxylin and eosin (H&E) with standard methods.

5. Real-Time quantitative polymerase chain reaction (qRT-PCR)

Total RNA from ear tissues was isolated with the Hybrid-R™ (GeneAll Biotechnology, Seoul, Korea) according to manufacturer's recommended protocol. cDNA was synthesized using PrimeScrip™ RT Master Mix (Takara, Shiga, Japan). IFN- γ (*Forward :5'-GATGCATTCATGAGTATTGCCAAGT-3'*, *Reverse: 5'-GAGGACCACTCGGATGAGCTC-3'*) and IL-4 (*Forward:5'-AGAT CATCGGCATTTTGAAC-3', Reverse: 5'-TTTGGCACATCCATCTCCG-3'*) gene expression were analyzed using ABI Step One Plus Real-Time qPCR machine (Applied Biosystems, Foster City, CA, USA) and expression level of gene

transcript was normalized to H-prt (*Forward 5'-CAGTCAACGGGGGACATAA-3', Reverse: 5'-GGGCTGTACTGCTTAACCA-3'*).

6. Statistical analysis

Data were analyzed with the unpaired Student's two-tailed t-test, unless otherwise stated, using the Prism software (GraphPad Software Inc). $P < 0.05$ was considered to be significant.

III. RESULTS

1. IL-4 was significantly increased during the sensitization phase of OXA-induced CHS murine model.

In the sensitization phase of C57BL/6 WT mice, mRNA levels of cytokines were analyzed in SDLNs at different time points after a single epicutaneous sensitization with OXA. The mRNA expression levels of IL-4, a major cytokine in the Th2 differentiation, was markedly increased starting from 72hr after the sensitization, while those of IFN-γ did not show significant increase (Fig. 1). These data suggest that in the sensitization phase of OXA-induced CHS model, Th2-type immunity seems to be a major immune response of T cell differentiation. Therefore, I sought to determine whether this type of immune response was also comparable in the elicitation phase and to investigate which DC subset played a major role in the priming of T cells responsible for acute CHS to OXA.

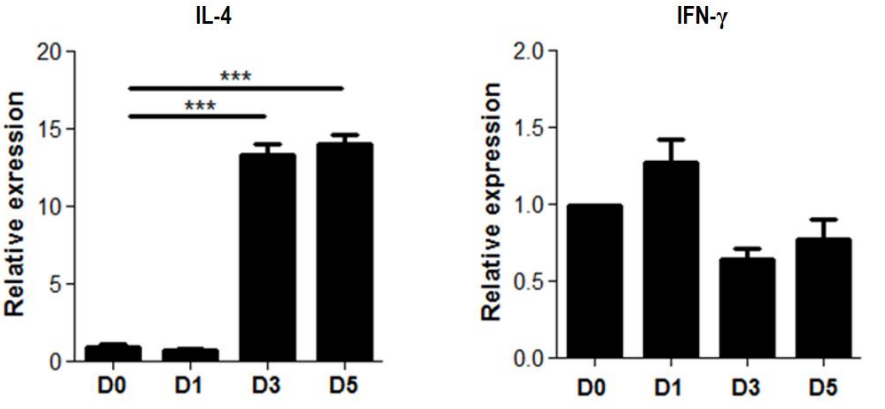


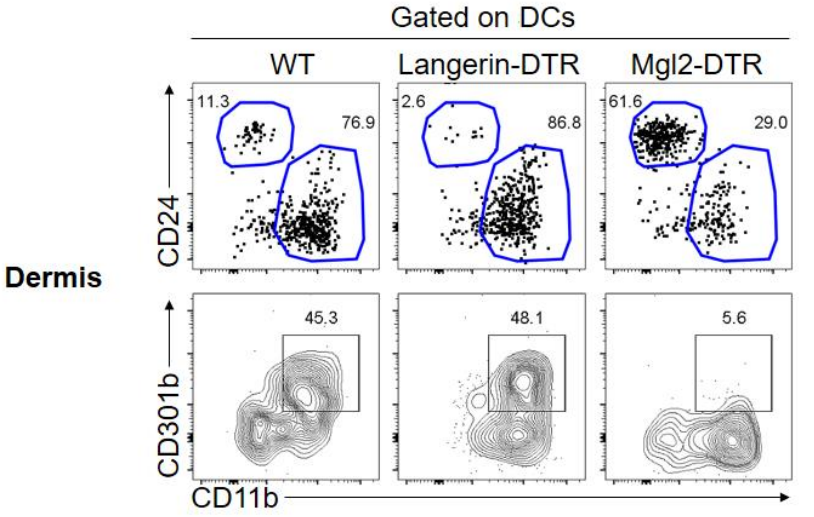
Figure 1. Relative expressions of mRNA of IL-4 and IFN-γ during the sensitization phase of OXA-induced CHS murine model.

On day 0, day 1, day 3, and day 5 after the sensitization with OXA, axillary SDLNs were analyzed by qRT-PCR.

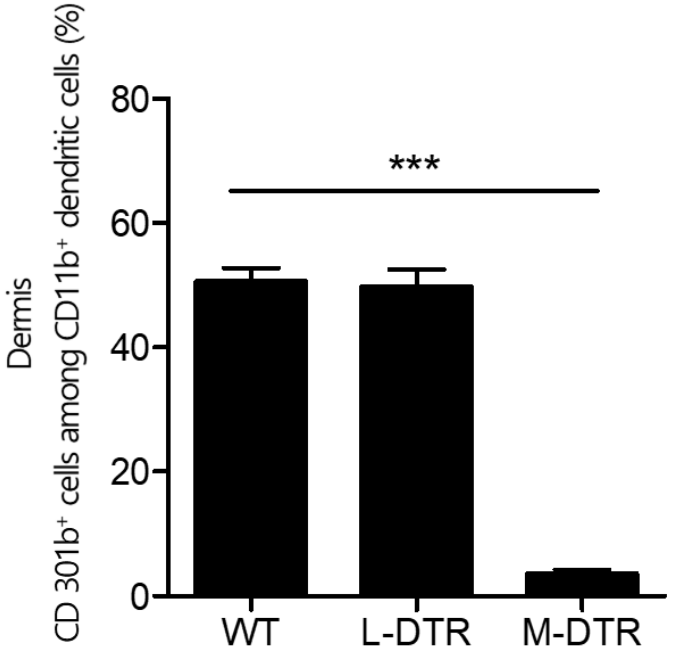
2. Specific DC subsets were depleted upon injection of diphtheria toxin (DT) in transgenic mice.

To identify the responsible DC subset, I used transgenic mice which can demonstrate in vivo environment lacking specific types of DCs. To selectively deplete CD301b$^+$ dDC in vivo, I used Mgl2-DTR mice. In Mgl2-DTR mice, a single intraperitoneal injection of DT selectively depleted CD301b$^+$ dDCs in the dermis analyzed by flow cytometry (Figs. 2A and 2B). For unknown reason, DT-treated Mgl2-DTR mice showed a partial depletion of epidermal LCs (Fig. 2C). Langerin-DTR mice were used to make another depletion model lacking Langerin$^+$ cells including LCs and Langerin$^+$ dDCs. Using flow cytometric analysis, I confirmed that epidermal LCs and dermal Langerin$^+$ dDCs were depleted in Langerin-DTR mice which underwent single intraperitoneal injection of DT (Figs. 2A and 2C).

A



B



C

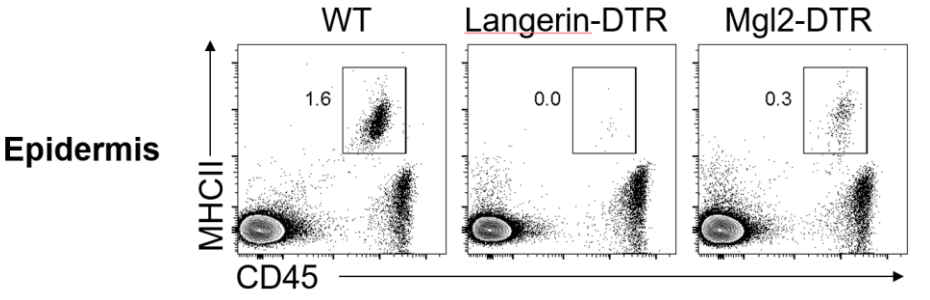


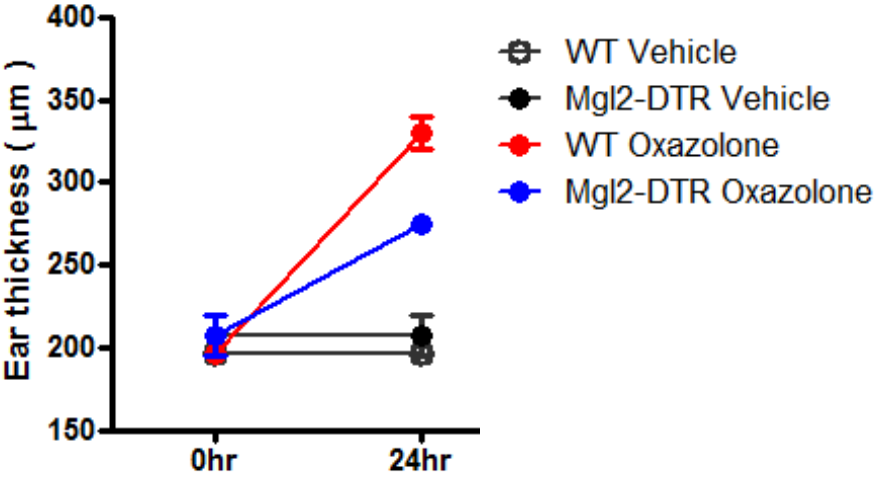
Figure 2. Depletion of specific DC subsets in DT-treated transgenic mice

(A) Dermal DCs 1 day after DT injection were gated for CD301b and Langerin$^+$ DC markers in flow cytometry. Among conventional DCs expressing CD11b, a portion of DCs expressing CD301b was depleted in Mgl2-DTR mice. Langerin$^+$ dDCs, which comprise a subset of conventional DCs expressing CD24, were depleted in Langerin-DTR mice. (B) In quantitative analysis of flow cytometry, a depletion of a portion of CD301b$^+$ dDCs among CD11b$^+$ cells was observed in Mgl2-DTR mice. (C) In flow cytometric analysis, cells of the epidermis were gated with CD45 and MHCII to detect the LCs in Langerin-DTR, Mgl2-DTR, and WT control mice.

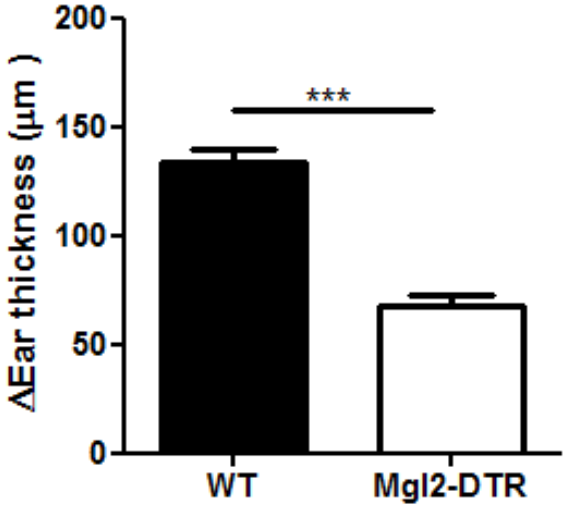
3. Mgl2-DTR mice showed reduced OXA-induced acute CHS.

To address whether CD301b$^+$ dDCs play a role in the OXA-induced acute CHS, WT and Mgl2-DTR mice were sensitized with OXA and treated with DT followed by challenging with OXA or vehicle. Vehicle-treated sides did not show a significant increase in ear thickness in both types of mice. For OXA-treated side, WT control mice displayed a significantly greater increase in ear thickness compared to Mgl2-DTR mice at 24hrs postchallenge (Figs. 3A and 3B). When examined histologically, ears from OXA-sensitized and challenged WT control mice displayed significant inflammatory cellular infiltrates and pronounced epidermal and dermal thickening that were markedly reduced in the ears from similarly treated Mgl2-DTR mice (Fig. 3C). Cellular infiltration was absent in vehicle-challenged ears of the mice in both genotypes. These data demonstrate a requirement of CD301b$^+$ dDCs in the development of an acute CHS inflammatory response to OXA.

A



B



C

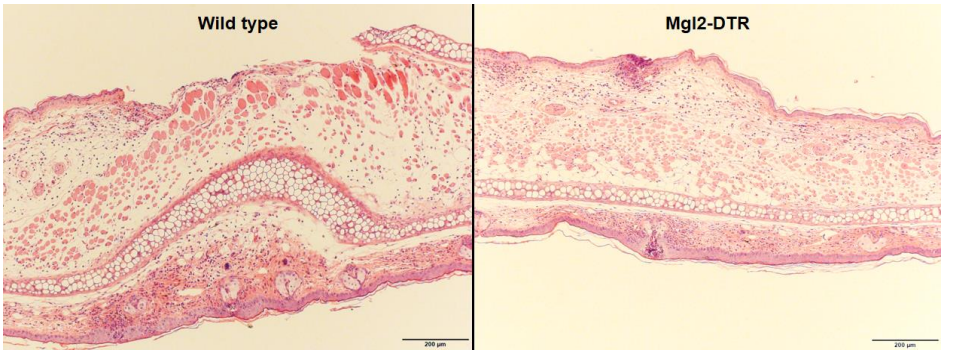


Figure 3. Reduced OXA-induced acute CHS responses in Mgl2-DTR mice

(A) The thickness of OXA- and vehicle-challenged ears were measured in DT-treated Mgl2-DTR and WT control mice. (B) Changes in ear thicknesses (ΔEar thickness) showed statistically significant reduction in DT-treated Mgl2-DTR mice compared to WT control mice. (C) Ear tissues of DT-treated Mgl2-DTR mice displayed markedly reduced inflammatory infiltrates and thickening in H&E stains compared to WT control mice.

4. Th2 responses were diminished in OXA-challenged Mgl2-DTR mice.

To identify the dominant type of T cell responses induced by CD301b$^+$ dDCs in the OXA-induced acute CHS, I analyzed the cytokine expressions in ear tissues of WT and Mgl2-DTR mice at 24hrs postchallenge. In WT mice, the expression of both IFN-γ and IL-4 significantly increased in OXA-treated side compared to vehicle-treated side. The extent of increase in relative mRNA expression of IL-4 was much higher than that of IFN-γ (Fig. 4). Consistent with the reduced Δear thickness and inflammatory infiltration, the level of IL-4 transcript was significantly reduced in the ear tissue of the OXA-challenged Mgl2-DTR mice compared to that of WT controls (Fig. 4). In contrast, IFN-γ expression of Mgl2-DTR mice did not show significant difference compared to that of WT mice. This indicates that CD301b$^+$ dDCs induces CHS response to OXA by priming Th2 cells, not Th1 cells.

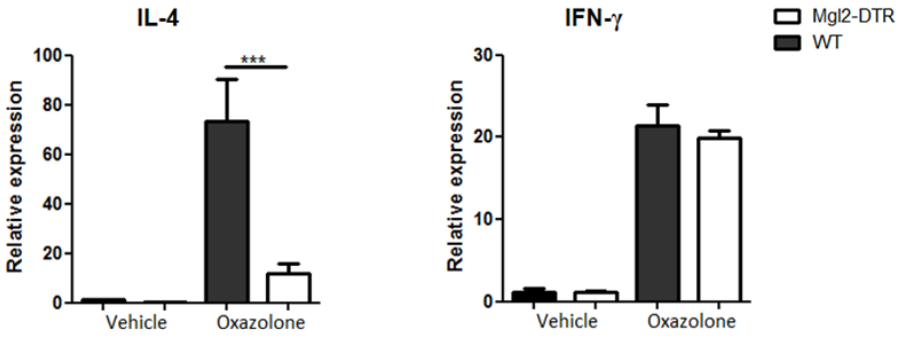


Figure 4. Diminished Th2 responses in elicitation phase of Mgl2-DTR mice

qRT-PCR of IL-4 and IFN-γ was prepared to evaluate the relative levels of the respective mRNA.

5. OXA-induced acute CHS response as well as cytokine expression pattern of Langerin-DTR mice was comparable to WT mice.

To determine whether LCs or Langerin$^+$ dDCs have a critical role in OXA-induced acute CHS responses, WT and Langerin-DTR mice were sensitized with OXA and treated with DT followed by challenging with OXA or vehicle. Ear thickness of Langerin-DTR mice showed comparable increase with WT mice (Fig. 5A). In addition, the mRNA expression of both IFN-γ and IL-4 in Langerin-DTR mice did not show significant difference compared to WT mice (Fig. 5B). Taken together, these data suggest that depletion of both LCs and Langerin$^+$ dDCs did not significantly compromise both Th1- and Th2-type immune responses, which might result in normal CHS response to OXA. In addition, these results further support the critical role of CD301b$^+$ dDCs in OXA-indcued CHS responses by excluding the possible effects of a partial depletion of epidermal LCs in Mgl2-DTR mice.

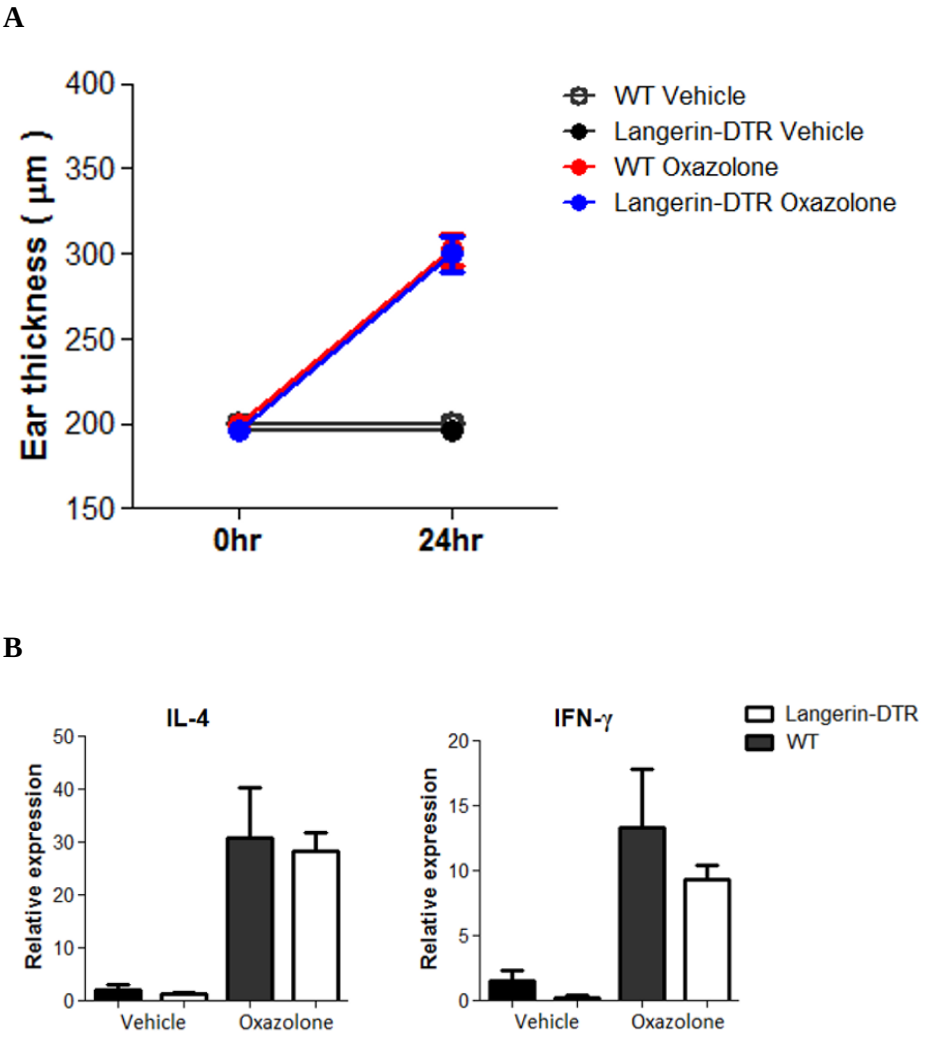


Figure 5. Ear thickness and mRNA expression of cytokines in Langerin-DTR mice.

(A) Ear thickness of OXA-treated side of Langerin-DTR mice showed similar increase with those of WT mice. (B) Relative expressions of IL-4 and IFN-γ transcript of DT-treated Langerin-DTR and WT control mice were measured from the respective ear samples by qRT-PCR.

IV. DISCUSSION

In this study, in order to investigate the exact pattern of T cell differentiation in OXA-induced acute CHS murine model, I analyzed profiles of cytokine expressions in sensitization and elicitation phase of wild type C57BL/6 mice which were sensitized and challenged to OXA.

After single epicutaneous sensitization with OXA, cytokine mRNA expressions in axillary SDLNs were analyzed with qRT-PCR on baseline, 1st, 3rd, and 5th day to identify the sequential pattern of T cell differentiation during the sensitization phase. I confirmed the marked increase of IL-4 starting from 72hr time point after sensitization, while significant increase or decrease of IFN-γ was not detected. Given these results, it is likely that OXA-induced CHS response is initiated by mainly Th2 cell priming in the sensitization phase.

In the elicitation phase, cytokine mRNA expressions in OXA-challenged ear tissue were analyzed and compared to vehicle-treated ear tissues to identify the pattern of T cell at 24hr after the hapten challenge. Significant increase in mRNA expressions of both IL-4 and IFN-γ was observed. Specifically, IL-4 transcripts increased with relatively higher extent than IFN-γ consistent with the changes in the sensitization phase showing significant increase in only IL-4 expressions.

Conventionally, as high levels of IFN-γ and an accumulation of Th1 cells were observed at the elicitation sites, it has been believed that murine CHS was mainly mediated by Th1 and regulated by Th2 cells.[4] Furthermore, typical haptens used in CHS model including TNCB, DNFB, DNCB, and OXA have generally been regarded as Th1 IFN-γ-inducing haptens.[6] A previous report demonstrated that repeated elicitation with hapten results in a shift from Th1- to Th2-mediated cutaneous inflammation.[19] Therefore, OXA has been used chronic CHS model or atopic dermatitis model to induce Th2-mediated responses only by repetitive multiple epicutaneous challenges.[20] A few previous studies using single epicutaneous OXA-challenging murine model, which showed mainly IFN-γ induction without significant increase of IL-4 expressions, support this hypothesis regarding OXA. Webb *et al*. presented that an initial challenge with

OXA led to a transient increase in TNF-α production followed by IFN-γ, while there was a minimal production of IL-4. Subsequently, continued exposure to OXA led to a downregulation of IFN-γ and an upregulation of IL-4 production.[19] Another study by Li *et al*. demonstrated significantly increased IFN-γ/IL-4 ratios compared to the baseline ratio in the acute phase of OXA-induced CHS.[21] One study which analyzed the cytokine profiles in bronchoalveolar lavage fluid of mice exposed to epicutaneous application of OXA also showed Th1-dominant immune responses in the acute phase.[22]

However, in this study, significant increase of IL-4 expression was observed during the sensitization phase, and both IFN-γ and IL-4 significantly increased at the elicitation phase after only single epicutaneous challenge with OXA. These results suggest that sensitization and challenge of OXA seems to stimulate both Th1 and Th2 immune responses in "acute phase" of CHS model. Repetitive epicutaneous challenges through several days may not be necessary to induce Th2-type CHS responses. This discrepant result compared to previous studies might be affected by the differences in the type of mice used and in the dose of OXA used in CHS model. Majority of previous studies described above used BALB/c mice, while this study used C57BL/6 mice as wild type mice, which might have influence in the pattern of T cell differentiation.[19-22] The concentrations of OXA and types of solvents were also slightly different among previous studies.

Given that the human ACD cannot be defined as a simple Th1-dominant immune response, the murine CHS model which is capable of inducing both type 1 and type 2 immune responses is of importance. One study which detected the differentially expressed genes in skin tissue of the patients with ACD by gene array analysis revealed that human ACD can be considered as heterogenous entities showing various immune polarization according to the allergens, but not as a single entity.[23] Specifically, fragrance and rubber, which are one of the most common allergens in human ACD, induced both Th1 and Th2 immune responses with a stronger Th2-biased response, of which an immune polarization is similar to that of OXA-induced murine CHS model in

my study. Nickel, in contrast, induced markedly Th1-dominant responses.[23] Taken together, in future researches, OXA-induced acute CHS model with C57BL/6 mice might be used as a simple animal model closely mimicking the immune polarization of human ACD induced by cosmetics or daily necessities other than metallic products.

CD301b$^+$ dDC is a recently described, unique subset of dDCs which has been proven to be phenotypically and functionally distinct from LCs or Langerin$^+$ dDCs and to be related to Th2 cell-mediated immunity.[24-26] Previous studies regarding association between Th2 immunity and CD301b$^+$ dDCs used FITC- or protein antigen-induced CHS model which were classically considered to provoke Th2 responses.[27-30] As Th2 immunity was observed as a major immune response in OXA-induced acute CHS model, I sought to investigate whether CD301b$^+$ dDCs play a critical role in skin elicitation induced by OXA via Th2 type immune response. I used Mgl2-DTR mice which was validated to be an in vivo depletion model lacking CD301b$^+$ dDCs in previous studies.[30,31] As expected, skin of Mgl2-DTR mice showed significant reduction of specific portion of dDCs featured by expressing CD11b and CD301b upon intraperitoneal injection of DT. Though ear thickness increased in OXA-treated side compared to vehicle-treated side of Mgl2-DTR mice, significantly less changes in ear thickness were observed compared to those of WT mice. This result indicates that although CD301b$^+$ dDC is not the only involved cells in inducing CHS responses to OXA, it seems to have a critical role. In addition, in Mgl2-DTR mice, mRNA level of IL-4 was significantly reduced compared to wild type mice, while IFN-γ did not show significant difference, which suggests that CD301b$^+$ dDCs may have a critical role in OXA-induced acute CHS model by inducing type 2 immunity, not type 1.

One possibility for the OXA-induced acute CHS is affected by other types of DC subsets, I used depletion model of LCs and Langerin$^+$ dDCs. In addition, as a partial depletion of epidermal LCs was observed in DT-treated Mgl2-DTR mice, the effect of LC-depletion had to be further investigated. Epidermal LCs and Langerin$^+$ dDCs are the two subsets of DCs that express CD207 (Langerin)

and thought to be important for differentiation of Th17 and Th1 cells, respectively.[32] It has been previously reported that skin-resident Langerin$^+$ DCs, including LCs and Langerin$^+$ dDCs, do not efficiently transport protein antigens coinjected with papain to the dLN and are therefore not responsible for the papain-induced Th2 cells.[33] My study revealed that LCs and Langerin$^+$ dDCs are also not responsible for the OXA-induced Th2 cell differentiation. Interestingly, mRNA expression of IFN-γ as well as those of IL-4 was not influenced by the depletion of LCs and Langerin$^+$ dDCs, which indicates that Langerin$^+$ DCs are not the exclusive immune cell subset contributing to the induction of Th1 responses to OXA. This result is consistent with a recent study which showed that antigen presentation by Langerin$^+$ dDCs alone did not appear to represent the main pathway involved in sensitization for CHS.[16]

Although I observed the diminished Th2 responses resulting in reduced OXA-induced CHS response, there was no evidence whether the reduction in Th1 response would lead to the reduced CHS. This relationship remains to be elucidated. In addition, whether the increase of IL-4 is mainly driven by Th2 cells, not by other types of immune cells such as basophils, and whether blockade of the IL-4 itself can lead to reduced CHS response are not demonstrated in this study. Furthermore, although Langerin-DTR mice were utilized in my study, a more sophisticated depletion model is necessary to completely exclude the effects of a partial depletion of LCs in DT-treated Mgl2-DTR mice. A further investigation of these issues is warranted in the future studies.

V. CONCLUSION

In conclusion, here I demonstrate that the OXA-induced acute CHS murine model accommodate dominant Th2 immunity but also increased Th1 immunity. This result suggests that OXA-induced acute CHS can be a simple murine model which requires both Th1 and Th2 immunity in the pathogenesis. In addition, my work clearly shows that Th2 immunity in the OXA-induced acute CHS model is mediated by the $CD301b^{+}$ dDC subset.

REFERENCES

1. Peiser M, Tralau T, Heidler J et al. Allergic contact dermatitis: epidemiology, molecular mechanisms, in vitro methods and regulatory aspects. Current knowledge assembled at an international workshop at BfR, Germany. Cell Mol Life Sci 2012;69:763–81.
2. Ale IS, Maibacht HA. Diagnostic approach in allergic and irritant contact dermatitis. Expert Rev Clin Immunol 2010;6:291-310.
3. Grabbe S, Schwarz T. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. Immunol Today 1998;19:37–44.
4. Honda T, Egawa G, Grabbe S, Kabashima K. Update of immune events in the murine contact hypersensitivity model: toward the understanding of allergic contact dermatitis. J Invest Dermatol 2013;133:303-15.
5. He D, Wu L, Kim HK, Li H, Elmets CA, Xu H. IL-17 and IFN-gamma mediate the elicitation of contact hypersensitivity responses by different mechanisms and both are required for optimal responses. J Immunol 2009;183:1463–70.
6. Larson RP, Zimmerli SC, Comeau MR, Itano A, Omori M, Iseki M, et al. Dibutyl phthalate-induced thymic stromal lymphopoietin is required for Th2 contact hypersensitivity responses. J Immunol 2010;184:2974–84.
7. Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K, Shiohara T. Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. J Immunol 1997;159:2484-91.
8. Tkalčević V, Cužić S, Kramarić MD, Parnham MJ, Eraković Haber V. Topical azithromycin and clarithromycin inhibit acute and chronic skin inflammation in sensitized mice, with apparent selectivity for Th2-mediated processes in delayed-type hypersensitivity. Inflammation 2012;35:192-205.

9. Segura E. Review of Mouse and Human Dendritic Cell Subsets. Methods Mol Biol 2016;1423:3-15.
10. Kissenpfennig A, Henri S, Dubois B, Laplace-Builhé C, Perrin P, Romani N, et al. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity 2005;22:643–54.
11. Bursch LS, Wang L, Igyarto B, Kissenpfennig A, Malissen B, Kaplan DH, et al. Identification of a novel population of Langerin^{+} dendritic cells. J Exp Med 2007;204:3147–56.
12. Wang L, Bursch LS, Kissenpfennig A, Malissen B, Jameson SC, Hogquist KA. Langerin expressing cells promote skin immune responses under defined conditions. J Immunol 2008;180:4722–7.
13. Romani N, Clausen BE, Stoitzner P. Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin. Immunol Rev 2010; 234: 120-41.
14. Gao Y, Nish SA, Jiang R, Hou L, Licona-Limon P, Weinstein JS, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. Immunity 2013;39:722-32
15. Kashem SW, Riedl MS, Yao C, Honda CN, Vulchanova L, Kaplan DH. Nociceptive Sensory Fibers Drive Interleukin-23 Production from CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity. Immunity 2015;43:515-26.
16. Honda T, Nakajima S, Egawa G, Ogasawara K, Malissen B, et al. Compensatory role of Langerhans cells and langerin-positive dermal dendritic cells in the sensitization phase of murine contact hypersensitivity. J Allergy Clin Immunol 2010;125:1154–56.
17. Noordegraaf M, Flacher V, Stoitzner P, Clausen BE. Functional redundancy of Langerhans cells and Langerin^{+} dermal dendritic cells in contact hypersensitivity. J Invest Dermatol 2010;130:2752–9.

18. Edelson BT, Kc W, Juang R. Peripheral CD103^{+} dendritic cells form a unified subset developmentally related to CD8alpha^{+} conventional dendritic cells. J Exp Med 2010;207:823–36.
19. Webb EF, Tzimas MN, Newsholme SJ, Griswold DE. Intralesional cytokines in chronic oxazolone-induced contact sensitivity suggest roles for tumor necrosis factor alpha and interleukin-4. J Invest Dermatol 1998;111:86-92.
20. Otsuka A, Nakajima S, Kubo M, Egawa G, Honda T, Kitoh A, et al. Basophils are required for the induction of Th2 immunity to haptens and peptide antigens. Nat Commun 2013;4:1739.
21. Li P, Zhao Y, Ge L. Therapeutic effects of human gingiva-derived mesenchymal stromal cells on murine contact hypersensitivity via prostaglandin E2-EP3 signaling. Stem Cell Res Ther 2016;7:103.
22. Mori T, Tanimoto Y, Ota M, Masakado T, Kitamoto S, Saito K, Isobe N, Kaneko H. Comparison of cytokine profiles in bronchoalveolar lavage fluid of mice exposed to respiratory and contact sensitizers. J Toxicol Sci 2012;37:337-43.
23. Dhingra N, Shemer A, Correa da Rosa J, Rozenblit M, Fuentes-Duculan J, Gittler JK, et al. Molecular profiling of contact dermatitis skin identifies allergen-dependent differences in immune response. J Allergy Clin Immunol 2014;134:362-72.
24. Tsuiji M, Fujimori M, Ohashi Y, Higashi N, Onami TM, Hedrick SM, et al. Molecular cloning and characterization of a novel mouse macrophage C-type lectin, mMGL2, which has a distinct carbohydrate specificity from mMGL1. J Biol Chem 2002;277:28892–901.
25. Onami TM, Lin MY, Page DM, Reynolds SA, Katayama CD. Generation of mice deficient for macrophage galactose- and N-acetylgalactosamine-specific lectin: limited role in lymphoid and erythroid homeostasis and evidence for multiple lectins. Mol Cell Biol 2002;22:5173–81.
26. Dupasquier M, Stoitzner P, Wan H, Cerqueira D, van Oudenaren A. The

dermal microenvironment induces the expression of the alternative activation marker CD301/mMGL in mononuclear phagocytes, independent of IL-4/IL-13 signaling. J Leukoc Biol 2006;80:838–49.

27. Kitajima M, Iwamura C, Miki-Hosokawa T, Shinoda K, Endo Y, Watanabe Y, et al. Enhanced Th2 cell differentiation and allergen-induced airway inflammation in Zfp35-deficient mice. J Immunol 2009;183:5388-96.
28. Kumamoto Y, Denda-Nagai K, Aida S, Higashi N, Irimura T. MGL2 Dermal dendritic cells are sufficient to initiate contact hypersensitivity in vivo. PLoS One 2009;4:e5619.
29. Murakami R, Denda-Nagai K, Hashimoto S, Nagai S, Hattori M, Irimura T. A unique dermal dendritic cell subset that skews the immune response toward Th2. PLoS One 2013;8:e73270.
30. Kumamoto Y, Linehan M, Weinstein JS, Laidlaw BJ, Craft JE, Iwasaki A. CD301b^{+} dermal dendritic cells drive T helper 2 cell-mediated immunity. Immunity 2013;39:733-43.
31. Ginhoux F, Collin MP, Bogunovic M, Abel M, Leboeuf M, Helft J, et al. Blood-derived dermal langerin^{+} dendritic cells survey the skin in the steady state. J Exp Med 2007;204:3133–46.
32. Kaplan DH, Igyarto BZ, Gaspari AA. Early immune events in the induction of allergic contact dermatitis. Nat Rev Immunol 2012;12:114–24.
33. Tang H, Cao W, Kasturi SP, Ravindran R, Nakaya HI, Kundu K, et al. The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. Nat Immunol 2010;11:608–17.

ABSTRACT(IN KOREAN)

접촉과민반응 마우스 모델에서 나타나는 제 2형 T세포 면역반응에 필수적인 피부수지상세포 아형에 대한 규명

<지도교수 이민걸>

연세대학교 대학원 의학과

이 재 원

알레르기성 접촉피부염은 가장 흔한 피부 질환 중 하나로, 전세계 인구의 15-20%가 경험하는 것으로 알려져 있다. 접촉과민반응 마우스 모델은 알레르기성 접촉피부염을 연구하기 위한 동물 모델 중 가장 흔히 사용되고 있는 모델이다. 특정 표면 마커를 가지고 특정 기능을 하는 새로운 아형의 진피 수지상세포들이 밝혀지고 있다. 그러나 각각의 수지상세포 아형이 접촉피부염에서 갖는 정확한 역할은 아직 정립되지 않은 상황이다. 특히, 접촉피부염 마우스 모델에서 나타나는 제 2형 T 세포 반응에 중요한 역할을 하는 수지상세포의 아형은 정확히 밝혀지지 않았다. 이 연구에서는 옥사졸론 유발 접촉피부염 마우스 모델에서 제 2형 T 세포 면역반응의 증가를 확인하고, 이러한 면역 반응에 관여하는 피부수지상세포의 아형이 무엇인지 밝히고자 하였다.

이번 연구에서는 50μl의 3% 옥사졸론을 쥐의 복부에 도포하여 감작시켰고, 유발기에서는 10μl의 0.6% 옥사졸론을 쥐의 왼쪽 귀에 도포하였다. 감작기에서 옥사졸론 감작 이후 림프절에서 사이토카인 mRNA 발현 정도를 분석하였다. 유발기에서는 옥사졸론 도포 후 24시간 이후에 귀 두께와 사이토카인 mRNA 발현을 각각 디프테리아 톡신으로 처리한 야생형 (wild type), Mgl2-DTR 유전자변형 마우스에서 분석하였다. 같은 방식의

접촉피부염 모델을 Langerin-DTR 마우스에서도 진행하였다.

감작기에서는 옥사졸론 도포 이후 72시간이 지난 시점부터 IL-4의 발현이 현저히 증가함을 확인하였고 IFN-γ의 경우에는 증가하거나 감소하지 않음을 확인하였다. 유발기에서는 야생형과 비교하여 Mgl2-DTR 마우스에서 귀 두께의 증가 정도가 현저히 적었으며 IL-4의 발현 정도도 낮음을 확인하였다. IFN-γ의 경우에는 야생형과 유의미한 차이를 보이지 않았다. 반면, Langerin-DTR 마우스에서는 옥사졸론 유발 접촉피부염의 정도 및 사이토카인 발현 정도가 야생형과 비교하여 유의미한 차이를 보이지 않았다.

이 연구 결과를 고려하였을 때, 제 2형 T 세포 면역반응이 옥사졸론 유발 접촉피부염 마우스 모델에서 핵심적이며, 이러한 면역반응을 유발하는데 $CD301b^{+}$ 진피 수지상세포가 중요한 역할을 하는 것으로 사료된다.

핵심되는 말: 알레르기성 접촉피부염, 접촉과민반응, 피부수지상세포, Mgl2-DTR 마우스, Langerin-DTR 마우스, 인터루킨-4, 피부면역학