Functional role of epidermal Langerhans cells in imiquimod-induced psoriasis-like dermatitis model

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Abstract

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Psoriasis is a chronic inflammatory skin disease, in which multiple immune mechanisms are involved. In psoriasis, the inflammatory cell infiltrate consists mainly of dendritic cells (DCs), macrophages and T cells in the dermis and neutrophils in the epidermis. The murine skin contains several types of DC subsets. Langerhans cells (LCs) are DCs that reside in the epidermis. The functional role of LCs has been extensively studied in the contact hypersensitivity model. However, only few studies have investigated on the role of LCs in psoriasis-like dermatitis model, but the results were variable and the exact role of epidermal LCs in psoriasis model remains to be elucidated. The aim of this study was to explore the functional role of LCs in imiquimod (IMQ)-induced psoriasis-like inflammation model using human langerin-diphtheria toxin active subunit (hLang-DTA) mice (LC depleted

mouse model).

In this study, 5% IMQ cream was topically applied on the ear and back skin of mice for 6 consecutive days. Clinical and histopathologic features were evaluated. Psoriasis related mRNA expression was analyzed by using quantitative RT-PCR from the ear and back skin and the expression of psoriasis related cells and production of cytokines including IL-17A and IL-22 were assessed by flow cytometry from skin and draining lymph nodes.

The results showed that hLang-DTA had reduced IMQ-induced psoriasis-like skin inflammation featuring erythema, scales and ear thickness compared with wild type mice. In skin and skin draining lymph nodes of hLang-DTA mouse, with significant LC depletion, infiltration of IL22-producing γδTCR cells were decreased. And LC-depleted skin showed decreased IL-22 mRNA induction by IMQ.

The findings of this study can suggest that LCs are required for the development of IMQ-induced psoriasis-like dermatitis by induction of IL-22-producing γδ T cells.

Key words: Psoriasis, Langerhans cell, Dendritic cell, hLang-DTA mouse, Interleukin 22, Cutaneous immunology

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I. INTRODUCTION

Psoriasis is an inflammatory epidermal hyperproliferative skin disease affecting 2-3% of the worldwide population. Clinically, it is characterized by erythematous and scaly plaques over the skin surface.[1] Psoriasis was originally thought to be a disease primarily of dysfunctional epidermal keratinocytes.[2] However, substantial studies have demonstrated that the cellular innate and adaptive immune responses, especially the T cell-mediated systems, play a crucial role in the pathogenesis of psoriasis.[3,4] In addition, cytokine network is important in psoriasis, and the expression levels of interleukin (IL)-1, tumor necrosis factor (TNF), IL-12, IL-17, IL-22, and IL-23 are elevated in human psoriatic skin.[5] Recently, the discovery of IL-23/Th17 axis in the pathophysiology of psoriasis shifted the cytokine paradigm from Th1 to Th17 cytokines, focusing mainly on IL-17 and IL-22. As Th17 cytokines, IL-17 and IL-22 activate and induce proliferation of keratinocytes in

psoriatic skin.[6]

The inflammatory cell infiltrate in psoriatic skin consists mainly of dendritic cells (DCs), macrophages and T cells in the dermis and neutrophils in the epidermis.[7] Among these immune cells, DCs are found to be markedly increased within psoriatic lesions.[8] Although DCs are suggested to have central roles in the pathogenesis of psoriasis, the specific role of each DCs are still unclear.[9]

DCs of the human skin can be categorized by the surface marker, location, and function. Langerhans cells (LCs), which are characterized by expression of Langerin and CD1a, are resident epidermal DCs and have well-defined role as antigen-presenting cells (APCs) in contact dermatitis.[10] For dermal DCs, there are CD1c positive resident dermal DCs, CD1c negative inflammatory DCs that produce IL-23 and induce and activate Th17 differentiation. Dendritic cell-lysosomal-associated membrane protein (DC-LAMP) or CD83 positive cells, which are thought to contribute to the chronic nature of psoriasis, sustain chronic T-cell activation.[9,11,12] In addition, plasmacytoid DCs regulate inflammation, link innate/adaptive immunity, and produce large amount of IFN-α and inflammatory dendritic epidermal cells (IDECs) with lower expression of CD1a, which are thought to be either monocyte-derived inflammatory DCs and are found to be markedly increased in epidermis of inflammatory skin disease such as psoriasis and atopic dermatitis.[13,14]

Unlike human skin, DCs of the murine skin can be largely categorized into 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 consist of two main subsets, namely Langerin$^+$ DCs, and Langerin$^-$ DCs, and the majority of dermal DCs are Langerin negative.[15] LCs can be distinguished from dermal Langerin$^+$ DCs by higher expression of CD11b, epithelial-cell adhesion molecule (EpCAM) and lower expression of CD103.[16]

The functional role of LCs has been extensively studied in the context of contact hypersensitivity model. It seems that LCs show both immunogenic or tolerogenic characteristics depending on the types of inflammatory models tested. [17,18]

In psoriatic patients, the role of LCs is currently unclear. The density of LCs has been shown to be decreased in psoriatic skin, whereas the numbers of LCs were similar to normal skin.[9,13] Also, the migration of LCs is found to be markedly impaired in non-lesional psoriatic epidermis, and the cytokines that affect LC migration are found to be different in early-onset and late-onset psoriasis.[19]

For psoriasis-like skin inflammation model in mice, imiquimod (IMQ)-induced model is commonly used. It has been demonstrated that topical application of IMQ, a ligand for Toll-like receptor (TLR) 7 and TLR8, induces psoriasis-like skin inflammation, inducing acanthosis, parakeratosis, and a mixed inflammatory

infiltrate.[20] Currently this model is used in several research groups to understand the underlying immune-pathogenesis of psoriasis. Furthermore, IMQ-induced skin inflammation is critically dependent on the IL-23/Th-17 axis and IMQ-treated mice show increased numbers of IL-17-producing γδ T cells.[21] All findings closely resemble human plaque type psoriasis.

To date there are few studies that explored the role of LCs in the setting of IMQ-induced psoriasis-like model. Some results suggest that LCs are required for the development of psoriasis-like lesions,[22] but other data support an anti-inflammatory role of LCs,[23] or that they are not critical for the initiation or regulation of the disease.[24] These discrepant data may result from the use of different LC-ablating strategies and immunological readouts. Thus, the exact role of epidermal LCs in the pathogenesis of psoriasis is still unclear and need to be elucidated further.

LC-depleting mouse models in previous studies were conducted by using murine Langerin-diphtheria toxin receptor (DTR) mice. Murine Langerin-DTR mice were generated by the introduction of the primate receptor for diphtheria toxin (DT) into the endogenous murine Langerin locus.[25,26] Injection of DT ablates all Langerin-expressing DCs and results in depletion of LCs. On the other hand, human Langerin-DTA (hLang-DTA) mice are transgenic mice, which express the diphtheria toxin active subunit (DTA), not the receptor, as a bacterial artificial chromosome transgene under the control of human Langerin genomic locus.[27] Expression is specifically limited to LCs which leads to their constitutive ablation,

while leaving all other cells including dermal Langerin^{+} DC intact.[28] Thus, using different form of transgenic mice (hLang-DTA mouse) compared with the previous studies, we hypothesize that this study can draw novel insights and consequentially provide further implications to understand the role of LCs in psoriasis-like skin inflammation. The aim of this study was to explore the functional role of LCs in IMQ-induced psoriasis-like inflammation model using hLang-DTA mice.

II. MATERIALS AND METHODS

1. Mice

Mice carrying transgene of human Langerin promoter-driven DTA (hLang-DTA mouse) were purchased from Jackson Laboratory (Bar Harbor, ME, USA). Age- and sex-matched littermate mice devoid of transgene were used as wild-type controls 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 Department of Laboratory Animal Resources Committee of Yonsei University College of Medicine.

2. Imiquimod-induced psorasiform dermatitis model

5% IMQ cream (Aldara; 3M pharmaceuticals, St. Paul, MN, USA) was topically applied on the ear or back skin of mice for 6 consecutive days as previously reported.[21] Skin inflammation severity of erythema, thickness, and scale was evaluated using a defined rating system (0: none, 1: mild, 2: moderate, 3: marked, 4: severe).

3. Tissue preparation and flow cytometry

Excised skin was digested in 1mg/ml collagenase IV (Worthington, Lakewood, CA, USA) for 1hr and passed through 40 μm strainer to generate single cell suspension. Lymph nodes were teased using forceps and similarly processed as skin. Surface staining was carried out using following antibodies (eBioscience, San Diego, CA, USA); CD4, CD11b, CD11c, CD24, CD103, CD324, αβTCR, γδTCR, I-A/I-E, IL-17, IL-22. For intracellular cytokine staining, cells were incubated for 6hr in complete medium containing leukocyte activation cocktail with Golgi Plug (BD Biosciences, San Jose, CA, USA), and then permeabilized with CytoFix/Perm (eBioscience).

4. Histopathology

Skin sections of 7-μm thickness were prepared with Cryostat (Leica, Wetzlar, Germany). Skin sections were stained with hematoxylin and eosin with standard methods.

5. Real-Time quantitative polymerase chain reaction

Total RNA was extracted using TRizol (Ambion, Carlsbad, CA, USA) and isopropanol precipitation method. A total of 1000ng of RNA was used for making cDNA using Reverse Transcription kit (Takara, Kusatsu, Japan). IL17a, IL23a, IL22, Tnfα, and Krt16 gene expressions were analyzed using ABI Real-Time qPCR machine (Applied Biosystems, Foster City, CA, USA). The primer sequences used in this study are listed in Table 1. Samples were held for 30sec at 95°C and amplified by 42 cycles of two-step PCR program at 95°C for 5sec and 60°C for 30 sec. Melting point analysis was carried out by heating the amplicon from 60 to 95°C.

Table 1. Primer sequences used for real-time qPCR

Genes	Forward	Reverse
Il17a	CAGCAGCGATCATCCCTCAAAG	CAGGACCAGGATCTCTTGCTG
Il22	ATGAGTTTTTCCCTTATGGGGAC	GCTGGAAGTTGGACACCTCAA
Il23a	GACCCACAAGGACTCAAGGA	CATGGGGCTATCAGGGAGTA
Krt16	CCACTCCTCCTCACAGCACTC	CCTGGAACTCTGACTTTGGCTCT
Tnfa	ATGTCCATTCCTGAGTTCTG	AATCTGGAAAGGTCTGAAGG
Gapdh	TGGCCTTCCGTGTTCCTAC	GAGTTGCTGTTGAAGTCGCA

6. Statistical analysis

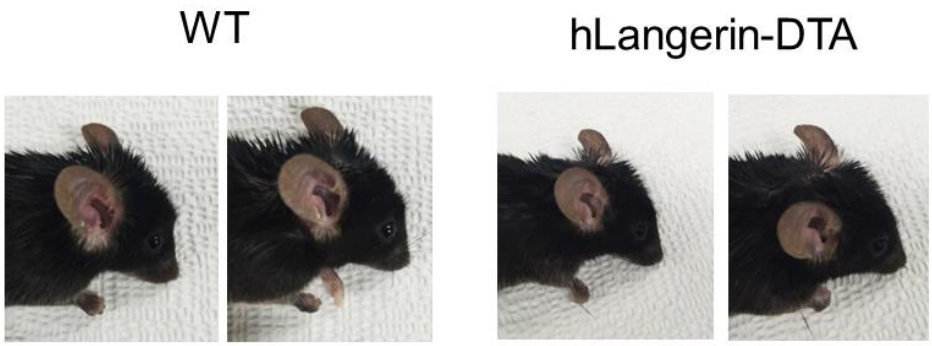
Data were analyzed with unpaired Student's two-tailed t-test, unless otherwise stated, using the Prism software (GraphPad Software Inc., San Diego, CA, USA). $P < 0.05$ was considered to be significant.

III. RESULTS

1. hLang-DTA mice show reduced IMQ-induced psoriasis-like skin inflammation

Both hLang-DTA mice and wild type mice showed psoriasis-like lesions featured by redness, scales and thickening after topical application of IMQ. Compared with IMQ-applied wild type mice, hLang-DTA mice showed reduced erythema, and scales (Fig. 1A~D). The difference was more distinct in back skin than ear skin of mice (Fig. 1A and Fig. 1C). Ear thickness in IMQ-treated hLang-DTA mice showed less increases compared with wild type mice (Fig. 1B). Histopathologically, IMQ application in wild type mice resulted in marked acanthosis, parakeratosis, loss of granular layer, structures mimicking Munro's microabscess in stratum corneum, and inflammatory cell infiltration, showing psoriasis-like features. On the other hand, IMQ application had fewer effects in hLang-DTA group, showing less acanthosis and parakeratosis. Also, granular layer was intact in this mice group and there were lack of a discrete Munro's microabscess (Fig. 1E). Furthermore, epidermal thickness was significantly decreased in hLang-DTA group compared with that of wild type (Fig. 1F) ($p<0.05$). These findings suggest that LCs are involved in the induction of IMQ-induced psoriasis-like skin inflammation.

(A)



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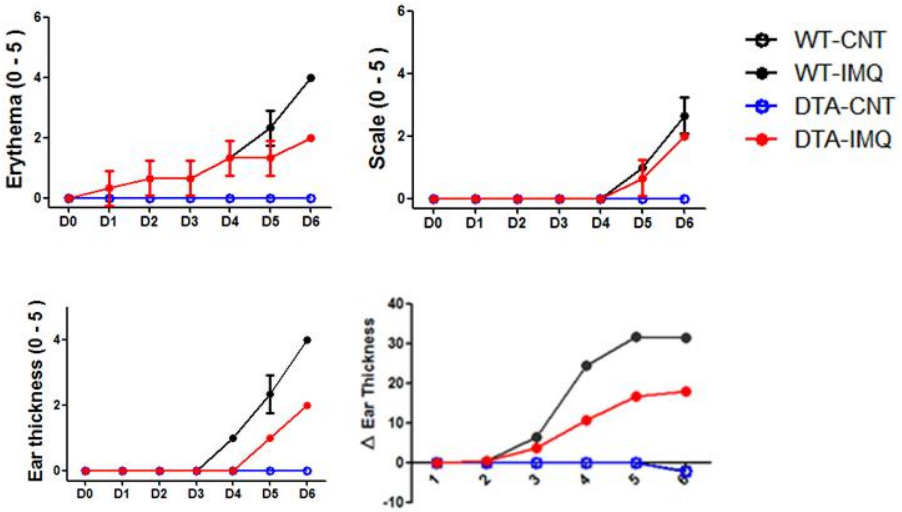

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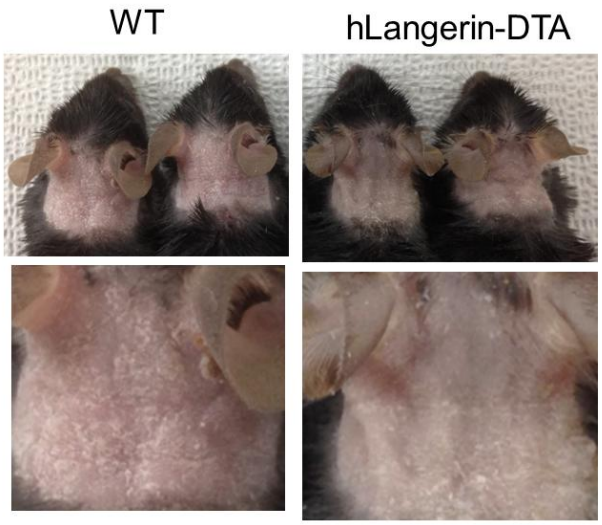
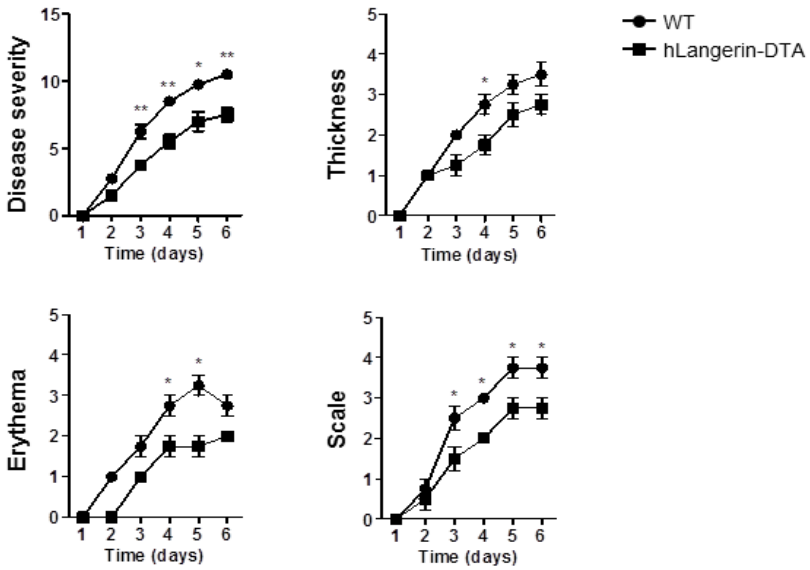


(D)



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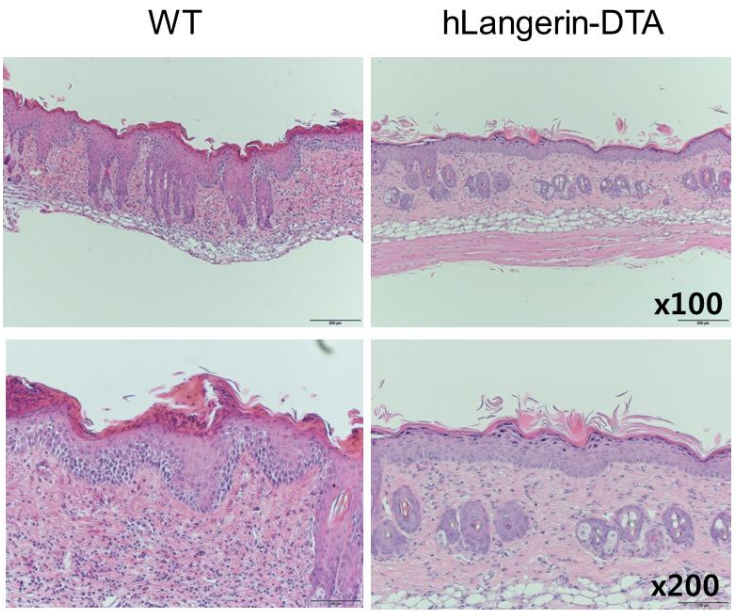

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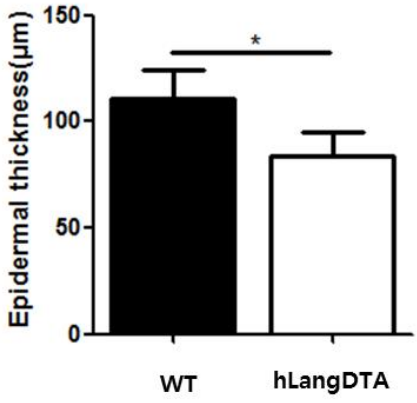


Figure 1. hLang-DTA mice showed reduced IMQ-induced psoriasis-like inflammation compared with wild type. (A) IMQ-induced psoriasis-like skin inflammation in ear skin, showing reduced redness, scales and swellings in hLang-DTA mice compared to the wild type mice. (B) Severity of IMQ-induced psoriasis-like skin inflammation is represented by the scoring system. Scores were lower in hLang-DTA mice compared with wild type mice. Ear thickness was reduced in hLang-DTA mice. (C) IMQ-induced psoriasis-like skin inflammation in back skin, redness and scales are considerably decreased in hLang-DTA mice. (D) Severity scores of back skin were lower in hLang-DTA mice. (E) Microscopy of cross-sections of back from mice, revealed by hematoxylin and eosin staining. IMQ-applied wild type mice showed marked acanthosis, parakeratosis, loss of granular layer. Structure like Munro's microabscess was found. hLang-DTA mice showed less acanthosis and parakeratosis. (F) Epidermal thickness was significantly decreased in hLang-DTA mice (*p <0.05).

†Abbreviations: WT, wild type; CNT, control; IMQ, imiquimod.

2. hLang-DTA mice showed significantly low LC populations compared to wild type, even when inflammation is induced by IMQ

To examine the extent of LC depletion in hLang-DTA mice, LC populations were analyzed from skin single cell suspensions using multi-color flow cytometry. LCs are characterized by $CD11c^{+}MHCII^{+}EpCAM^{+}CD103^{-}$ immunophenotype. Even when inflammation was induced by IMQ, $EpCAM^{+}CD103^{-}$ cells, which could be thought as LCs, showed significantly low populations when compared with wild type. In control, without IMQ application, these cells were almost not detected in hLang-DTA mice. However, 7.6% of $EpCAM^{+}$, $CD103^{-}$ cells were detected even in hLang-DTA group (Fig. 2).

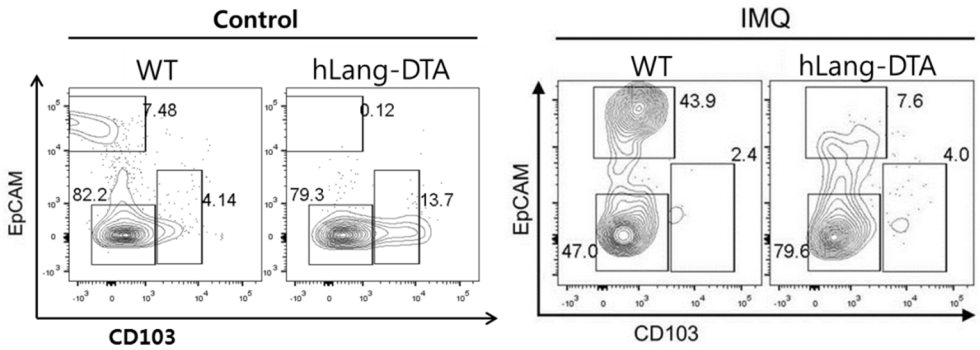


Figure 2. hLang-DTA mice showed significantly low LC populations compared to wild type in IMQ-induced inflammation status. Flow cytometric analysis of skin showed a significant decrease in $EpCAM^{+}CD103^{-}$ cell populations by 7.6%, which could be thought as LCs, in hLang-DTA mice compared with wild type, when inflammation was induced by IMQ application. In control group, $EpCAM^{+}$, $CD103^{-}$ cells were nearly absent by 0.12%.

†Abbreviations: LC, Langerhans cell; WT, wild type; IMQ, imiquimod.

3. hLang-DTA mice showed a decreased IL-22 mRNA expression in IMQ-induced psoriatic inflammation

To describe immune reactions in IMQ-induced psoriasis-like skin inflammation, mRNA expression of major cytokines in psoriasis was evaluated. In both wild type and hLang-DTA mice, IMQ application induced IL-23p19, IL-17a, IL-22 and Krt16 mRNA expressions in local skin. hLang-DTA group showed a significant decrease in IL-22 mRNA induction by IMQ application compared with wild type. Also, IL-23p19, IL17a and Krt16 mRNA levels had a tendency to be reduced in LC-depleted group, but these differences were not statistically significant (Fig. 3).

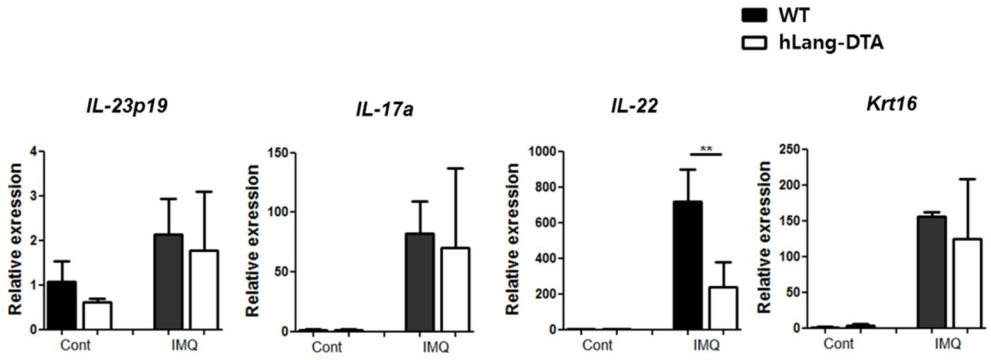


Figure 3. Skin of hLang-DTA mice showed a decreased IL-22 mRNA expression compared to wild type after IMQ application. IL-23p19, IL17a and Krt16 mRNA level were decreased in hLang-DTA mice after IMQ application. Among them, IL-22 mRNA expression showed significant reduction compared with wild type (**p<0.05).

†Abbreviations: IMQ, imiquimod.

4. IL-22 was mainly produced by dermal γδ T cells after IMQ application

To determine the cellular source of IL-22 in IMQ-induced inflammation status, cells were categorized by four groups, based on expression of TCRγδ and TCRβ. Cells were classified to epidermal γδ T cells with high TCRγδ expression, dermal γδ T cells with low TCRγδ expression, $TCR\gamma\delta^{-}TCR\beta^{-}$ non T lymphocyte and $TCR\beta^{+}$ CD4/CD8 T cells. Result showed that IL-22 was predominantly produced by dermal γδ T cells with low TCRγδ expression (Fig. 4).

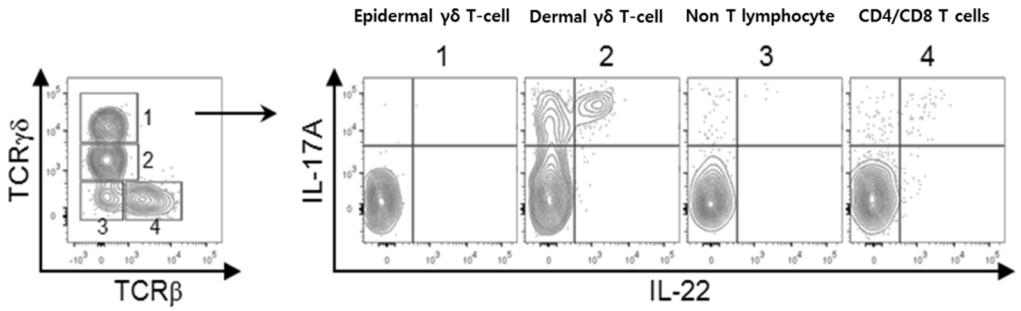


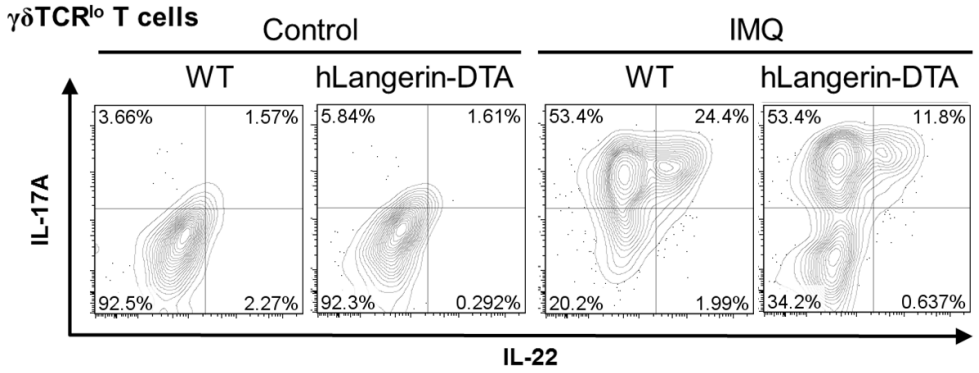
Figure 4. Major cellular source of IL-22 was dermal γδ T cells in IMQ-induced inflammation status. Flow cytometric analysis of skin showed four distinct cellular groups depending on expression of TCRγδ and TCRβ; Epidermal γδ T cell, dermal γδ T cell, non T lymphocyte and CD4/CD8 T cells. IL-22 was mainly produced by dermal γδ T cells.

†Abbreviations: IMQ, imiquimod.

5. Decreased infiltration of IL-22-producing γδ T cells in skin and skin draining LNs of hLang-DTA mice after IMQ application

Next, the production of IL-17A and IL-22 from dermal γδ T cells were investigated, which are shown to be major producers of IL-17 and IL-22 in this model. In the skin, when IMQ was applied, both wild type and LC-depleted mice group showed an increased IL-17A and IL-22 producing γδ T cell population. Compared with wild type, hLang-DTA group showed decreased number of IL-17A$^+$ and IL22$^+$ γδ T cells. However, only IL-22 producing γδ T cells showed a significant reduction in hLang-DTA mice compared to wild type with IMQ application (Fig. 5A and 5B). And it was shown that the total number of dermal γδ T cells was similar in both groups (Fig. 5C). In analysis of skin draining LNs, the number of IL-22$^+$ γδ T cells was significantly decreased in hLang-DTA group, but differences in IL-17$^+$ γδ T cells were not significant (Fig. 6A and 6B).

(A)



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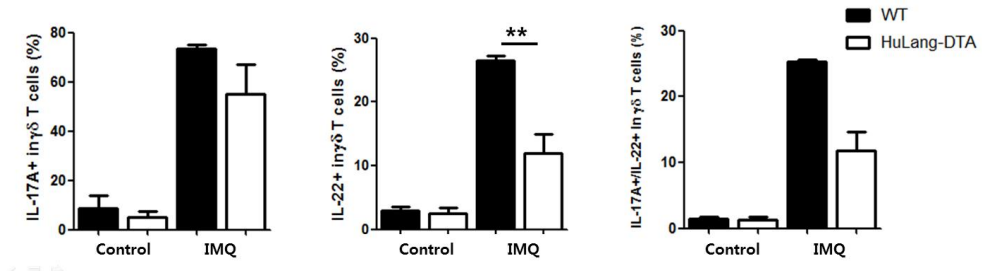

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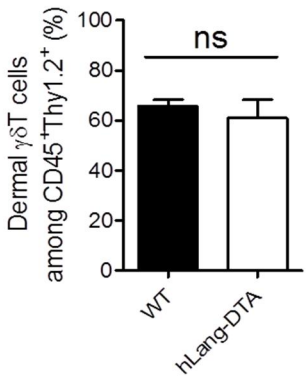
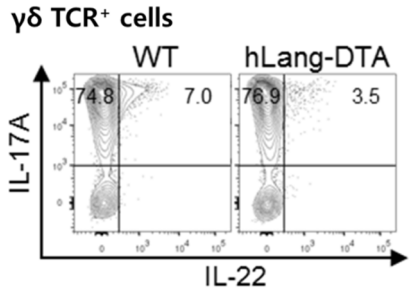


Figure 5. IL-22 producing γδ T cells are reduced in the skin of hLang-DTA mice after IMQ application. (A) Flow cytometric analysis of whole-skin suspension revealed that IL-17A^{+}, IL-22^{+} and IL-17A/22^{+} γδ TCR^{lo} cells were decreased in hLang-DTA mice compared with wild type. (B) Number of IL-22^{+} γδ TCR^{lo} cells was significantly decreased in hLang-DTA mice compared to wild type mice (**p<0.05). (C) Total number of dermal γδ T cells was similar in both wild type and hLang-DTA group after IMQ application.

†Abbreviations: WT, wild type; IMQ, imiquimod.

(A)



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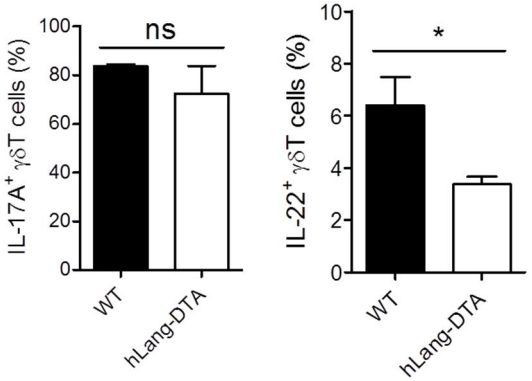

Figure 6. IL-22 producing γδ T cells are reduced in the skin draining LNs of hLang-DTA mice after IMQ application. (A) Flow cytometric analysis of skin draining LNs showed decreased IL-17A$^+$ and IL-22$^+$ γδ TCR$^+$ cells in hLang-DTA mice. (B) Number of IL-22$^+$ γδ TCR$^+$ cells were significantly decreased in hLang-DTA mice compared to wild type

†Abbreviations: WT, wild type; IMQ, imiquimod; LN, lymph node.

IV. DISCUSSION

In this study, in order to investigate the functional role of LCs in IMQ-induced psoriasis-like inflammation model, a constitutive LC depletion mouse model was used. Primarily, the ablation of LCs in hLang-DTA mice was verified. Then, the major inflammatory phenotypes of IMQ-induced inflammation in hLang-DTA mice were compared with wild-type mice.

The IMQ application resulted in increased erythema, scales and thickening of skins resembling psoriatic skin lesions. Also, histopathologic finding showed psoriasis-like changes characterized by marked acanthosis, parakeratosis and loss of granular layer.

As expected, skin of hLang-DTA mice showed significant reduction of LCs featured by $EpCAM^{+}$ and $CD103^{-}$ when inflammation was induced by IMQ compared to wild type. Though, LCs were nearly ablated in hLang-DTA mice in the steady state, around 7.6% of $EpCAM^{+}CD103^{-}$ cells were detected after IMQ-application. Interestingly, these cells expressed a relatively lower level of surface EpCAM compared to the resident LCs. In the inflammatory settings, it has been shown that monocytes with high expression of Ly-6C/G could differentiate into LCs *in vivo*.[29] Accordingly, these cell populations are thought to be inflammatory dendritic epidermal cells which are monocyte-derived inflammatory DCs. In order to confirm the exact type of these cells, additional analyses using monocyte marker such as Ly-6C/G are required in the future.

In previous studies, the investigation of the role of LCs in psoriasis-like dermatitis model with LC-depletion mice using Langerin-DTR mouse had variable results. It has been reported that after long-term application of IMQ, depletion of LCs resulted in increased ear thickness, erythema and scaling during late phase inflammation. It was also associated with increased neutrophil infiltration and extended more pustular lesions, suggesting anti-inflammatory role of LCs during psoriasis-like inflammation.[23] In another study using Langerin-DTR strain, mice lacking all Langerin$^+$ DCs developed a similar degree of psoriasiform inflammation as wild type, indicating a nonessential role of Langerin$^+$ cDCs, including LCs, during the initiation of IMQ-induced skin inflammation.[24] Meanwhile, there is a report that LC depletion resulted in attenuated IMQ-induced psoriasis-like dermatitis with reduced numbers of IL-17A$^+$ γδ T cells, suggesting LCs are main cutaneous DC subset in the development of IMQ-induced psoriatic lesions.

In our results, hLang-DTA mice, which are LC-depleted mice, showed considerably reduced IMQ-induced psoriasis-like inflammation featured by erythema, scales and thickening of skins compared with wild type. Also, histopathologic review revealed intact granular layer and decreased acanthosis and parakeratosis. Especially, epidermal thickness was significantly decreased compared with wild type. In addition, hLang-DTA group showed significantly decreased local mRNA expression level of IL-22 in IMQ-applied skin lesions.

Moreover, the IMQ-applied skin and skin draining LNs of hLang-DTA mice showed reduced number of IL-22 producing γδ TCR^+ cells.

IL-22 is a member of IL-10 family, which promotes the production of antimicrobial peptides, induces keratinocyte proliferation, epidermal hyperplasia, and inhibits terminal differentiation of keratinocytes. It is critically involved in pathologic characteristics of psoriasis.[30] In addition, studies using IL-22 knock out mice showed that IL-22 deficiency resulted in reduced psoriasis-like inflammation compared with wild type mice, suggesting that IL-22 is required for psoriasis-like lesions in mouse model.[31,32] In this study, IL-22mRNA and IL-22 producing γδ T cells were decreased in hLang-DTA group, and as a result, may have caused the decrease of acanthosis and parakeratosis. Cytokines including IL-23 are reported to induce the production of IL-22 by Th17/22 cells.[33] In addition to Th17 and Th22 cells, γδ T cells are also known to produce IL-22.[30] Also, there is a result that IL-23 induced $CCR6^+$ γδ T cells are major producers of IL-22 in a murine model of psoriasiform dermatitis.[34] Interestingly, an *in vitro* study of the human skin has found that human LCs efficiently induced IL-22 producing $CD4^+$ T-cells.[35] This report may support the finding in our study that LC is involved in the induction of IL-22 from T cells. Furthermore, it has been recently reported that activation of LCs by human β-defensin 3 sustained and amplified an IMQ-induced inflammation in mice.[36] However, currently it is not clear how murine LCs specifically regulate dermal γδ T cells to produce IL-22 upon IMQ application. It has been shown that

aryl hydrocarbon receptor transcription factor is needed for the *Il22* gene transcriptional activation in CD4+ T cells, but not in γδ T cells.[37] Thus, further study will be required to dissect the molecular mechanisms of LCs which affect IL-22 production from the various types of T cells.

Although IMQ-induced psoriasis-like skin lesions in mice share some clinical and histological characteristics with human psoriasis, there are several differences between them.[38] Intriguingly, a study using mice with transgenic expression of human CD1a showed that CD1a on LCs induced inflammation in psoriasis-like mice model. Also, in an in vitro analysis of human cells, anti-CD1a antibody reduced the level of IL-17A and IL22.[39] These results, taken together with our study, suggest evidence that LCs have a role in the development of IMQ-induced psoriasis-like inflammation, not only in psoriasis-like skin model of mouse but also in actual psoriasis patients.

V. CONCLUSION

As conclusion, the findings in this study demonstrated that LC depletion resulted in attenuated IMQ-induced psoriasis-like dermatitis with decreased numbers of infiltrating IL-22 producing γδ TCR cells and reduced IL-22 mRNA induction. These results suggest that LCs are required for the development of IMQ-induced psoriasis-like dermatitis by induction of IL-22-producing γδ T cells and their expression of IL-22 in mice. The induction of IL-22^+ γδ T cell by LCs is a novel finding that has not been reported previously, and IL-22^+ γδ T cells only, not IL-17^+ γδ T cells, were found to be decreased with LC depletion, in which further studies are needed to elucidate the exact mechanisms.

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ABSTRACT(IN KOREAN)

이미퀴모드 유발 건선양 피부염 모델에서의 표피 랑게르한스 세포의 역할 규명

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이 민 석

건선은 세계 인구의 약 2-3%에서 나타나는 표피 과증식 질환이다. 건선에서 염증세포의 침윤은 진피에서 수지상세포, 대식세포, T 세포가 관찰되고, 표피에서는 주로 호중구가 침윤 된다. 쥐의 피부에는 몇가지 형태의 수지상 세포가 존재한다. 랑게르한스 세포는 표피에 상주하는 수지상 세포이다. 랑게르한스 세포의 역할에 대한 연구는 접촉 피부염 모델에서 많이 이루어지고 있으나, 아직까지 건선에서 랑게르한스 세포의 역할은 확실하지 않다. 최근 건선양 피부염 모델에서 랑게르한스 세포의 역할을 규명하기 위한 몇가지 연구가 진행 되었지만, 아직까지 일정한 결과를 보이지 않았으며, 여전히 랑게르한스 세포의 역할은 명확하지 않은 상태이다. 따라서, 이 연구의 목표는 랑게르한스 세포가 결여 된 hLang-DTA 마우스를 사용하여, 이미퀴모드 유발 건선양 피부염 모델에서의 랑게르한스 세포의 역할을 규명 하고자 하였다.

본 연구에서는 5% 이미퀴모드 크림을 쥐의 귀와 등에 6일동안 연속적으로 도포하여 건선양 피부염을 유발시켰다. 임상적, 조직학적 소견을 평가하였고, 건선에 중요하다고 알려진 사이토카인인 IL-17A 와 IL-22를 분비하는 세포를 flow cytometry를 사용하여 분석하였다. 또한, RT-PCR을 통하여 피부에서의 mRNA의 발현정도를 분석하였다.

hLang-DTA 쥐는 야생형 (wild type) 에 비해서 이미퀴모드 유발 건선양 피부염이 감소 하였으며, 실제로 이미퀴모드로 염증을 유발한 상태에서도 hLang-DTA 쥐에서 랑게르한스 세포가 많이 줄어들어 있는 것을 확인하였다. 또한, hLang-DTA 쥐에서 IL-22를 분비하는 γδ T 세포들이 유의미하게 감소하였으며, IL-22 mRNA 발현도 유의미한 감소를 보였다.

이러한 결과 들을 통해서 랑게르한스 세포가 IL-22 분비 γδ T 세포를 유도하고, IL-22의 발현을 증가시킴에 따라, 이미퀴모드 유발 건선양 피부병변을 유발하는 데에 필요할 것이라고 사료 된다.

핵심되는 말: 건선, 랑게르한스 세포, 수지상 세포, 인터루킨 22, 피부 면역학