Pellino-1-dependent regulatory mechanisms of dendritic cell in *Candida albicans*-induced interleukin-17 immune response

Jeyun Park

Department of Medical Science
The Graduate School, Yonsei University

Pellino-1-dependent regulatory mechanisms of dendritic cell in *Candida albicans*-induced interleukin-17 immune response

Jeyun Park

Department of Medical Science
The Graduate School, Yonsei University

Pellino-1-dependent regulatory mechanisms of dendritic cell in *Candida albicans*-induced interleukin-17 immune response

Directed by Professor Min-Geol Lee

The Doctoral Dissertation
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requireme0ts for the degree
of Doctor of Philosophy

Jeyun Park

December 2020

This certifies that the Doctoral Dissertation of Jeyun Park is approved.

Thesis Supervisor: Min-Geol Lee

Thesis Committee Chair: Jeon-Soo Shin

Thesis Committee Member: Chae Gyu Park

Thesis Committee Member: Sun Och Yoon

Thesis Committee Member: Sung Jae Shin

The Graduate School
Yonsei University

December 2020

"There are no mistakes in life, just lessons "

Ziad K. Abdelnour

ACKNOWLEDGEMENTS

First of all, I want to express my greatest thanks to my supervisor, Professor Min-Geol Lee, who has supported and encouraged me during my doctoral study. His enthusiasm and guidance made me a better researcher. I can't thank him enough for his consideration.

I am also deeply grateful to my committee members, Professors Jeon-Soo Shin, Chae Gyu Park, Sun Och Yoon, and Sung Jae Shin, who took the time out of their busy schedules to give me helpful advice and comments. They were a big help to my doctoral thesis.

My gratitude goes to Joohee Lee, Lihua Che and Jongwook Oh for their wonderful help. I'm grateful to Tae-Gyun Kim for his advice and support during my doctoral studies. Also, the encouragement and generous assistance from my superior Sung Hee Kim have been of great help to me over the past 6 years.

I would like to say thank you to Jimin, Hee Sue, Myeonggil, Young Hun Kim, and Hongmin Kim, whom I met in the laboratory. They generously supported me in various ways. Also, thanks to my old friends, BP, Jim, Byul, and Sangyoun for always standing by my side. They were my delight and joy throughout the hard times of my laboratory life.

Last but not least, I wish to thank my family with all my heart. It is thanks to their love and warm consideration, that I could finish my dissertation. I don't think I'll ever forget every single thing they've done for me. Thanks again.

Jeyun Park

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLE

ABSTRACT

Pellino1-dependent regulatory mechanisms of dendritic cell in *Candida albicans*-induced interleukin-17 immune response

Jeyun Park

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Min-Geol Lee)

Dendritic cells (DCs) are strong antigen-presenting cells that are pivotal for the differentiation of naïve $CD4^+$ T cells into diverse effector T cells. Among T helper (Th) cells, Th17 cells, which produce members of the interleukin-17 (IL-17), are functionally involved in the host defense against extracellular pathogens and play a pathogenic role in various disorders. The pathogenesis of these disorders is closely linked with the defects in ubiquitination regulation. Pellino-1 (Peli1) is an E3 ubiquitin ligase which is the most specific enzyme in the ubiquitination system; this enzyme regulates various immune responses via ubiquitin-dependent mechanism. Although several studies have been performed using systemic Peli1-deficient mouse models, the functional role of Peli1 in DCs is not completely clear. Accordingly,

investigating the role of Peli1 in a cell-specific context may reveal its role in regulation of the immune responses. Thus, this study was conducted to determine the role of Peli1 within DCs by focusing on the Th17/IL-17 immune response. As a representative Th17 skin infection model, we used *Candida albicans*-mediated delayed type hypersensitivity (hereafter referred as the *C. albicans*-DTH model) induced by footpad injection.

By sensitizing *C. albicans*, immunological alterations in the form of elevated numbers of Th17 cells and IL-17 production were observed. Most importantly, the *C. albicans*-DTH model displayed an antigen-specific IL-17 DTH response following re-exposure to antigen. By using DC-specific Peli1 conditional knockout (cKO) mouse, we found that Peli1 in specific DC subsets controls the *C. albicans*-specific IL-17-producing $CD4^+$ T cell response. Additionally, analysis of Peli1-deficient bone marrow-derived DCs suggested that Peli1 in DCs regulates the production of cytokines critical for the differentiation of Th17 cells.

We revealed that Peli1 within DCs other than $Langerin^+$ DCs is critically involved in controlling *C. albicans*-induced Th17/IL-17 immune response in a cytokine-dependent manner. Thus, targeting Peli1 in certain DCs, which regulates Th17 cell differentiation, can be used to treat invasive *C. albicans* infections.

Key words: pellino-1, dendritic cell, interleukin-17, $CD4^+$ T cell, *Candida albicans*

Pellino-1-dependent regulatory mechanisms of dendritic cell in *Candida albicans*-induced interleukin-17 immune response

Jeyun Park

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Min-Geol Lee)

I. INTRODUCTION

Skin dendritic cells (DCs) are the first line of defense in protecting a host against foreign pathogens. In the skin, there are at least three subsets of DCs including epidermal Langerhans cell (LC), type 1 conventional DC (cDC1) and type 2 conventional DC (cDC2) in the dermis[1,2]. After exposure to antigens, DCs mature and migrate to draining lymph nodes (LNs) where they present antigens to T cells[3,4]. Among the antigen-presenting cells, DCs are the most effective at presenting antigens to naïve T cells and priming them to initiate the T cell-mediated adaptive immune response[5,6]. Naïve $CD4^+$ T cells are activated by stimulation of T cell receptor with antigen-major histocompatibility complex (MHC) molecules (Signal 1) and through interactions between costimulatory molecules on DCs and their ligands on T cells (Signal 2). Additionally, depending largely on the cytokine milieu secreted by DCs (Signal 3), $CD4^+$ T cells differentiate into specific T helper (Th) cells, including Th1, Th2, and Th17

cells, which show differences in cytokine production and function profiles[6,7].

Among Th cells, Th17 cells are characterized by the production of interleukin-17 (IL-17) family members. Th17 cells contribute to the host defense against extracellular pathogens and are functionally linked with several autoimmune diseases[7]. In addition, recent studies suggested that Th17 cells play a critical role in the pathogenesis of diverse diseases, including psoriasis, rheumatoid arthritis, cancer, inflammatory bowel disease, multiple sclerosis, asthma, bacterial infections, and fungal infections[8,9]. Particularly, there is increasing evidence that Th17-mediated immune disorders are associated with deregulation of ubiquitination[10,11].

Ubiquitination, defined as the attachment of ubiquitin (Ub) moieties to substrate proteins, is an important post-translational modification that modulates diverse biological processes such as cell cycle, proliferation, apoptosis, transcription, differentiation, gene expression, signal transmission, damage repair, and immune responses. It involves the sequential activity of various enzymes including activating (E1), conjugating (E2), and ligating (E3) enzymes[12]. As targeting E3 ubiquitin ligases is the most effective and specific therapeutic intervention in the ubiquitination system, several compounds targeting E3 ligases have been developed[13,14]. As E3 ubiquitin ligases, the Pellino (Peli) family was initially discovered to interact with *Drosophila* Pelle, an IL-1 receptor-associated kinase homolog. Members of the mammalian Peli family, namely Peli1, Peli2, and Peli3, have structural similarities, a conserved pattern of C-terminal RING-like domain, which are characteristic feature of E3 ubiquitin ligase[15-17]. However, unlike Peli2 and Peli3, Peli1 is highly expressed in lymphocytes including microglia, B cells,

and T cells[18,19]. Several studies of Peli1 revealed its regulatory role in microglial cells, B cells, and T cells using systemic Peli1-deficent mouse. Using a lupus-like disease model, Peli1 was found to play a role in regulating non-canonical nuclear factor-κB signaling pathway through lysine-linked ubiquitination in B cells[20]. Recent studies showed that Peli1 negatively regulates the activation of T cells through lysine 48-linked ubiquitination and Peli1-deficient mouse showed hyperactivation of T cells[18]. However, the results of previous functional studies are rather limited to understanding of the exact role of cell-specific Peli1, as systemic Peli1-deficient mouse models were used. Accordingly, additional investigations into the cellular function of Peli1 using conditional deletion of Peli1 in specific cell-type are strongly needed to further understand its function. Moreover, the effects of Peli1 within DCs in the context of helper T cell immunity are poorly understood, although various Th17/IL-17 immune-mediated diseases are known to be closely associated with deregulation of ubiquitination.

As a preliminary study, the capability of Peli1-deficient DCs for differentiating T cells was screened. The capacity of Peli1-deficient DCs towards IL-17-producing $CD4^+$ T cells was increased, which is consistent with the results of another study showing that Peli1 plays a role in the Th17-dependent experimental autoimmune encephalomyelitis model using systemic Peli1-deficient mouse[19]. Taken together, these results strongly suggest that Peli1 is involved in Th17-dependent immune response. Therefore, to identify the role of Peli1 in DCs during the Th17/IL-17 immune response, we used a *Candida albicans* model which is a representative model of Th17 skin infection.

Footpad injections are a convenient, relatively objective, sensitive, and simple method for assessing immune responses[21]. In this study, a *C. albicans*-mediated delayed type hypersensitivity (DTH) model (hereafter referred as the *C. albicans*-DTH model) was established by footpad injection.

Candida albicans is a commensal yeast species in humans causing opportunistic fungal infections known as candidiasis in the vagina, oropharynx, and skin. Many different *C. albicans* infection models commonly exhibit IL-17 immunity, and IL-17 is a key cytokine in the host defense against candidiasis[22,23]. Similarly, in the *C. albicans*-DTH model used in the current study, markedly increased Th17/IL-17 immunity was observed. Thus, we examined how Peli1 in DCs functions during *C. albicans*-induced Th17 immune response using cell-specific Peli1 conditional knockout (cKO) mouse. This study would highlight the possibility of manipulating Peli1 a promising therapeutic approach for controlling Th17/IL-17 immunity against severe invasive *C. albicans* infection.

II. MATERIALS AND METHODS

1. Mice

Wild-type (WT) C57BL/6 mouse was purchased from Orient Bio (Seongnam, Korea). Langerin-DTR mouse was a gift from Heung Kyu Lee at Korea Advanced Institute of Science and Technology (KAIST). CD301b-DTR mice was kindly provided by Akiko Iwasaki at Yale University. TEα mouse expressing a rearranged T cell receptor, specific for a short peptide derived from the *H2-Eα* gene (Eα52-68), was purchased from Jackson Laboratory (Bar Harbor, ME, USA). Mouse carrying the targeted *Pellino-1* (*Peli1)* allele, $Peli1^{tm1a(EUCOMM)Wtsi}$, was purchased from the Knockout Mouse Project Repository. To delete the neomycin-resistance cassette and generate mouse with a *Peli1*-null allele ($Peli1^{-/-}$, $Peli1^{tm1b}$), $Peli1^{tm1a(EUCOMM)Wtsi}$ mouse was bred with CMV-Cre C57BL/6 mouse which was a gift from Chul Hoon Kim at Yonsei University. Mouse with a *loxP*-flanked *Peli1* allele ($Peli1^{fl/fl}$, $Peli1^{tm1c}$) was generated by crossing $Peli1^{tm1a(EUCOMM)Wtsi}$ mouse with ACT-FLPe C57BL/6 mouse, which was a gift from Chul Hoon Kim at Yonsei University. To generate the systemic Peli1-deficienct mouse ($Peli1^{tm1d}$, Peli1 KO), $Peli1^{-/-}$ ($Peli1^{tm1b}$) mouse was bred with $Peli1^{fl/fl}$ ($Peli1^{tm1c}$) mouse because decreased survival during neonatal period was observed in the $Peli^{tm1b}$ mice possibly due to reduced care behaviors of $Peli^{tm1b}$ mother. CD11c-Cre mouse was gifted from Heung Kyu Lee at KAIST. To generate the DC-specific Peli1-deficienct mouse (CD11c-cKO), CD11c-Cre mouse was bred with $Peli1^{fl/fl}$ ($Peli1^{tm1c}$) mouse. To generate the LC-specific Peli1-deficienct mouse (huLang-cKO), human

Langerin-iCre mouse (huLangerin-iCre), which was a gift from Hyoung-Pyo Kim at Yonsei University, was crossed with *Peli1*$^{fl/fl}$ (*Peli1*^{tm1c}) mouse. Six-8-week-old mice, bred and housed under pathogen-free conditions in the animal facilities of the Yonsei University College of Medicine, were used for all experiments. This study was approved by the Institutional Animal Care and Use Committee (IACUC, Approval ID: 2019-0164).

2. Preparation of *C. albicans*

An isolated colony of *C. albicans* strain SC5314 on an agar plate was grown in YPAD medium overnight at 30℃. After washing in sterile phosphate buffered saline (PBS), the *C. albicans* was prepared at a final concentration of 1×10^7 in 40 μL PBS per footpad. For preparation of heat-killed (HK) *C. albicans*, *C. albicans* was grown in YPAD medium at 30℃ and then, *C. albicans* were killed by heating at 65℃ for 60 min.

3. Model establishment of *C. albicans*-induced delayed type hpersensitivity

To establish *C. albicans*-DTH model, mice were sensitized with *C. albicans* at a final concentration of 1×10^7 in 40 μL PBS per footpad. Seven days later, the mice were challenged with HK *C. albicans* at a final concentration of 1×10^7 in 40 μL PBS per footpad (Figure 2A). Mice were euthanized and skin-draining popliteal LNs were harvested on day 7 after the sensitization. For

clinical evaluation of DTH response, footpad thickness was measured using a dial thickness gauge (Peacock, Ozaki MFG Co. Ltd., Tokyo, Japan) and lesional footpad tissues were harvested at 24 hr after the challenge for further analysis.

4. Diphtheria toxin-induced DC depletion *in vivo*

Both Langerin-DTR and CD301b-DTR mice were treated intraperitoneally with 1 μg diphtheria toxin (DT, Sigma, St. Louis, MO, USA) dissolved in PBS at 1 day prior to and 1 day after *C. albicans* sensitization to deplete specific DC subsets. Specific subsets of DC were confirmed to be depleted from the epidermal and dermal cell suspensions at 24 hr after DT treatment.

5. Histological analysis

For histological examination, mouse footpad tissues and feet under the tarsus, including the foot bones, were harvested. After fixing these samples in 4% formalin solution (T&I, Chuncheon, Korea) for 24 hr, the tissues were embedded in paraffin, sectioned and stained with hematoxylin and eosin according to the manufacturer's protocol. Specimens were decalcified as needed with 0.1 M pH 7.4 EDTA solution (Invitrogen, Carlsbad, CA, USA) for 2 weeks after fixation and then fixed again in 4% formalin solution for 24 hr. The stained sections were analyzed by optical microscope for assessment.

6. Bone marrow-derived DC culture and stimulation

Whole bone marrow (BM) cells from the femurs and tibias of WT and CD11c-cKO mice were cultured in complete RPMI medium, which consisted of RPMI 1640 (Gibco, Grand Island, NY, USA) that was supplemented with 10% heat-inactivated fetal bovine serum (FBS, Welgene, Gyeongsan, korea), hydroxyethyl piperazine ethane sulfonic acid (HEPES, Gibco), and penicillin (Gibco), with granulocyte macrophage colony-stimulating factor (GM-CSF, JW CreaGene, Seongnam, Korea) and 2-mercaptoethanol (Gibco), for 7 days to generate BM-derived DCs (BMDCs). Half of the culture medium was removed and replenished with fresh medium containing GM-CSF and 2-mercaptoethanol every other day. On day 7, BMDCs were purified with beads-conjugated anti-CD11c mAb, a specific marker of BMDCs (Miltenyi Biotec, Bergisch Gladbach, Germany). The BMDCs were stimulated with 1×10^7 HK *C. albicans* /ml for 1–24 hr. The cells were then harvested for further analysis.

7. *In vivo* T cell proliferation and polarization assay

The transgenic T cell receptor of $CD4^+$ T cells in TEα mouse recognizes the Eα peptide. Splenic $CD4^+$ T cells from TEα mouse were purified using beads-conjugated anti-CD4 mAb (Miltenyi Biotec) and these cells were stained with 0.5 mM CellTrace™ Violet (CTV) for 10 min at 37℃. Isolated $CD11c^+$ BMDCs from WT and CD11c-cKO mice were stimulated with Eα peptide for 1 hr as described above. CTV-labeled $CD4^+$ T cells were co-cultured with WT or

Peli1-deficient stimulators with cytokines and neutralizing antibodies for 7 days at a DC: T cell ratio of 1: 5. For Th1 polarization, IL-2, IL-12 and αIL-4 were additionally added; For Th2 polarization, IL-4, αIFN-γ and αIL-12 were additionally added; IL-6, human transforming growth factor-β1 (hTGF-β1), IL-1β, αIFN-γ and αIL-4 were added for Th17 polarization. For flow cytometric analysis, cells were stimulated and stained as described below.

8. Quantitative real-time polymerase chain reaction

Total RNA from lesional footpad tissues and BMDCs non-stimulated or stimulated with HK *C. albicans* was isolated with a Hybrid-R total RNA kit (GeneAll Biotechnology, Seoul, Korea). cDNA was synthesized using PrimeScript™ RT Master Mix (Takara Bio, Shiga, Japan). Quantitative real-time polymerase chain reaction (real-time qPCR) was performed with the ABI StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA, USA) by monitoring the synthesis of double-stranded DNA during PCR cycles using SYBR Green (Takara Bio). The results were normalized to the level of *Hprt* mRNA. Primer sequences are listed in Table 1.

Table 1. Primer sequences used for real-time qPCR

Genes	Forward	Reverse
Hprt	TCAGTCAACGGGGGACATAAA	GGGGCTGTACTGCTTAACCAG
Il1b	TGACGGACCCCAAAAGATGA	GCTCTTGTTGATGTGCTGCT
Il4	AGATCATCGGCATTTTGAACG	TTTGGCACATCCATCTCCG
Il23a	GACCCACACAAGGACTCAAGGA	CAYGGGGCTATCAGGGAGTA
Il6	GCCAGAGTCCTTCAGAGAGA	GGAGAGCATTGGAAATTGGGG
Il17a	CAGCAGCGATCATCCCTCAAAG	CAGGACCAGGATCTCTTGCTG
Ifng	GATGCATTCATGAGTATTGCCAAGT	GTGGACCACTCGGATGAGCTC
Tgfb	CGCAACAACGCCATCTATGA	ACTGCTTCCCGAATGTCTGA

9. Cell isolation and flow cytometric analysis

Popliteal LNs were obtained on day 7 after *C. albicans* sensitization, and single-cell suspensions were obtained by passing total cells through a 70 μm nylon mesh filter. The cells were washed with PBS and erythrocytes were removed using red blood cell lysis buffer. For stimulation, cells were incubated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA , Sigma), and 500 ng/ml ionomycin (Sigma). After 1 hr, 10 μg/ml brefeldin A (BD Biosciences, San Jose, California, USA) and monensin (BD Biosciences) were added to the mixture and cultured for 5 hr. Cells were stained with the following fluorochrome-conjugated antibodies: CD3 (17A2), CD4 (GK1.5), TCRβ (H57-597), TCR Vα2 (B20.1), IFN-γ (PE-Cy7), IL-17A (TC11-18H10.1), MHCII (M5/114.15.2) from BioLegend (San Diego, California, USA); CD11c (N418), CD44 (IM7), IL-4 (11B11) from eBioscience (San Diego, California, USA); CD8a (53.6.7), CD80 (16-10A1) from BD Biosciences. The stained cells were analyzed using the FACS Verse or LSR Fortessa flow cytometer (BD Biosciences). All flow cytometry data were analyzed using the FlowJo software (Treestar, Ashland, Oregon, USA).

10. Enzyme-linked immunosorbent assay

Isolated $CD11c^+$ BMDCs were stimulated with 1×10^7 of HK *C. albicans* /ml for 24 hr as described above. IL-1β, IL-6 and TGF-β levels from the supernatant of BMDCs were measured with an enzyme-linked immunosorbent

assay (ELISA) according to the manufacturer's instructions (Mouse IL-1β and Human/Mouse TGF-β1 from eBioscience; Mouse IL-6 from BD Biosciences; IL-23 from Invitrogen).

11. **Statistical analysis**

Data were analyzed using an unpaired Student's two-tailed t-test or Mann-Whitney test and one-way analysis of variance (ANOVA) with Tukey's post-hoc test using Prism software (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically significant with $p < 0.05$.

III. RESULTS

1. Peli1-deficient DCs exhibited Th17-skewed immune response

Preferentially, the ability of WT and Peli1-deficient DCs for T cell differentiation was analyzed. For screening of T cell polarization, $CD4^+$ T cells of TEα mice after CTV labeling were co-cultured with WT or Peli1-deficient BMDCs, stimulated with Eα peptide, with cytokines for Th1, Th2, or Th17 cell differentiation. After 7 days, $CD4^+$ T cells were fully proliferated following co-culture with stimulators treated with Eα peptide (Figure 1A). Importantly, a skewed Th17 cell differentiation, but not the Th1 or Th2 cell differentiation, by co-culture with Peli1-deficient DCs was observed (Figure 1B). These findings suggested that Peli1 plays a role in Th17-dependent immune response, and therefore we focused on Th17/IL-17 immunity in the present study.

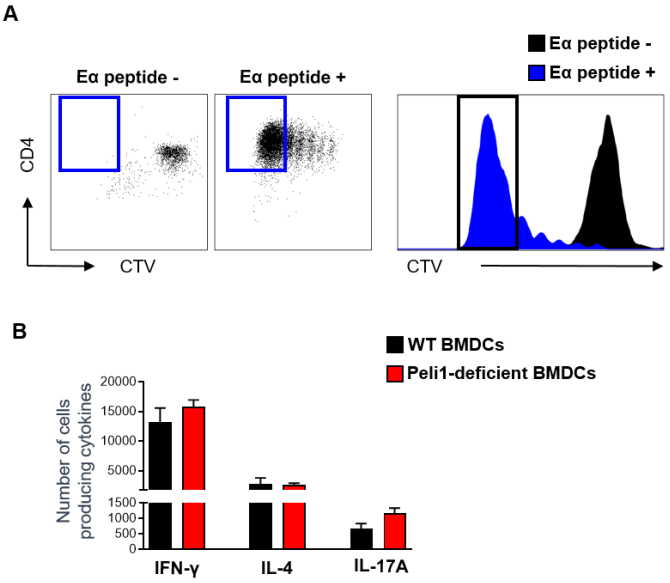

Figure 1. The polarizing capability of WT and Peli1-deficient BMDCs. (A) *In vitro* T cell polarization assay was performed using WT and Peli1-deficient BMDCs. CTV-labeled $CD4^+$ T cells of TEα mice were co-cultured with WT or Peli1-deficient BMDCs, stimulated with Eα peptide, with cytokines. Proliferation and polarization of $CD4^+$ T cells were analyzed by flow cytometry on day 7. Proliferation of $CD4^+$ T cells after 7 days marked with dilution of CTV is shown. (B) The numbers of IFN-γ-, IL-4-, and IL-17A-producing cells were analyzed in fully proliferated $CD4^+$ T cells. Error bars indicate mean ± standard error of the mean.

2. The *C. albicans*-DTH model was established by footpad injection of *C. albicans*

To study Th17/IL-17 immunity, a *C. albicans*-DTH model, which is a representative model of Th17 skin infections, was established via footpad injection. Specifically, the footpads of WT mice were sensitized with 2×10^7 *C. albicans.* After 7 days, the mice were challenged with 2×10^7 HK *C. albicans*. To examine local inflammation, the popliteal LNs, known as foot-draining LNs, were analyzed on day 7 after *C. albicans* sensitization. Twenty-four hours after *C. albicans* challenge, the antigen-specific DTH response in the footpad skin was analyzed (Figure 2).

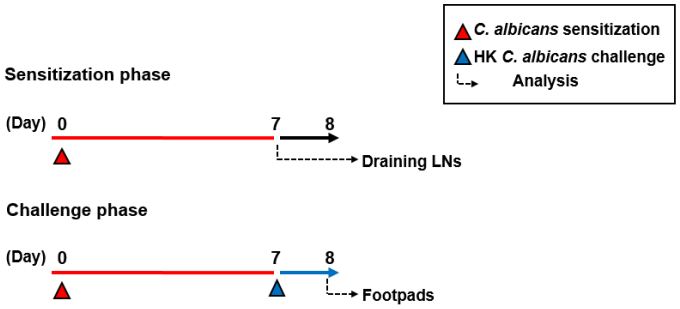

Figure 2. Establishment of the *C. albicans*-delayed type hypersensitivity model by footpad injection. The protocol for establishment of the *C. albicans*-DTH model is shown. The footpad skin of mice was sensitized with 2×10^7 *C. albicans*. After 7 days, mice were challenged with 2×10^7 HK *C. albicans* and DTH response was measured 24 hr after the challenge. HK, heat-killed.

3. The number of IL-17-producing $CD4^+$ T cells were increased in popliteal LNs following *C. albicans* sensitization

After *C. albicans* sensitization of footpad, the popliteal LNs, which were small in naïve mice, were enlarged (Figure 3A). In these popliteal LNs, the number of effector $CD4^+$ T cells notably increased whereas the number of effector $CD8^+$ T cells decreased 7 days after *C. albicans* sensitization. These immunological changes also increased the ratio of $CD4^+$ to $CD8^+$ T cells (Figure 3B, C). In the steady state, IL-17A was rarely observed in effector $CD4^+$ and effector $CD8^+$ T cells. Following *C. albicans* sensitization, effector $CD4^+$ T cells abundantly produced IL-17A whereas effector $CD8^+$ T cells still produced low amounts of IL-17A at day 7 (Figure 3C, D). These results demonstrated that IL-17-producing-$CD4^+$ T cells, not $CD8^+$ T cells, mainly function in the *C. albicans*-DTH model.

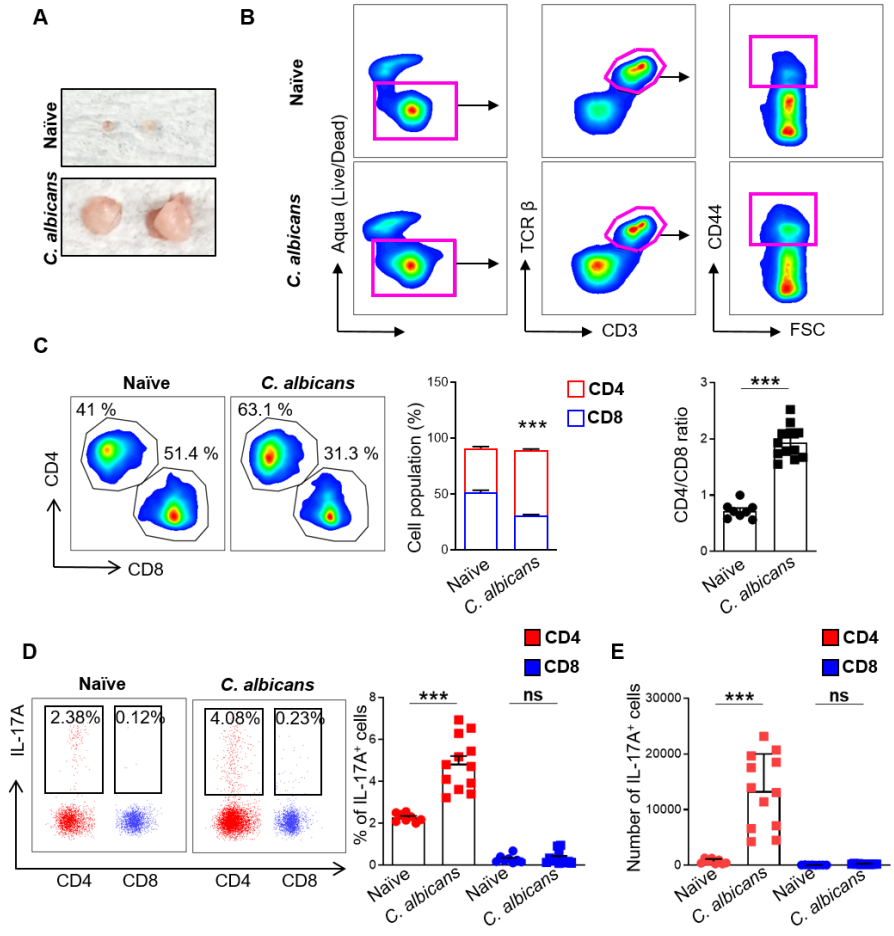

Figure 3. Increased numbers of IL-17-producing CD4$^+$ T cells in popliteal LNs after *C. albicans* sensitization. (A) Images of popliteal LNs of naïve and *C. albicans*-sensitized WT mice 7 days after the sensitization are shown. (B) Immunological changes in T cells of naïve and *C. albicans*-sensitized WT mice were examined using flow cytometry after 7 days. Gating strategies for the effector T cells of popliteal LNs are shown. Live cells were gated on CD3ε$^+$TCRβ$^+$T cells and CD44$^+$ to identify effector T cells. (C) The population

frequencies of $CD4^+$ and $CD8^+$ T cells following *C. albicans* sensitization are shown. The proportion of effector $CD4^+$ and $CD8^+$ T cells and the ratios of $CD4^+$ to $CD8^+$ T cells were also assessed. (D) The intracellular IL-17A production among effector $CD4^+$ and $CD8^+$ T cells was analyzed in naïve and *C. albicans*-sensitized WT mice. The proportion and frequencies of IL-17A-producing cells among $CD4^+$ and $CD8^+$ T cells are shown. (E) The absolute numbers of IL-17A-producing cells among effector $CD4^+$ T cells and $CD8^+$ T cells in naïve and *C. albicans*-sensitized WT mice are shown. Data were from at least two independent experiments with three to four mice per group. Error bars indicate mean ± standard error of the mean. ***, $p<0.001$; ns, not significant.

4. The *C. albicans*-DTH model displayed an antigen-specific IL-17 DTH response

C. albicans challenge after the sensitization led to local inflammation compared with naïve mice. At 24 hr after *C. albicans* challenge, mice exhibited intense footpad swelling (Figure 4A, B) Hematoxylin and eosin images of the foot region under the tarsus showed increased inflammatory cellular infiltration (Figure 4C).

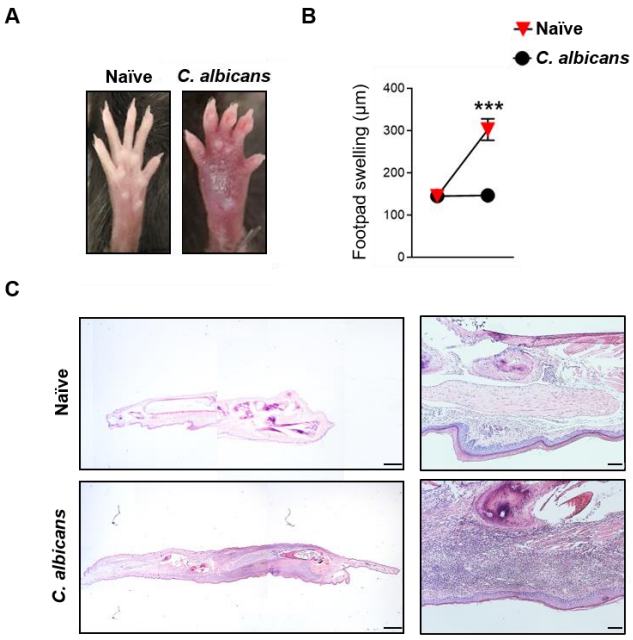

Figure 4. Local inflammation in the *C. albicans*-induced delayed type hypersensitivity model following *C. albicans* challenge. (A) Images of footpads of naïve and *C. albicans*-challenged WT mice and (B) changes in footpad swelling response 24 hr after *C. albicans* challenge are shown. (C) Representative hematoxylin and eosin images of the region under the tarsus of naïve and *C. albicans*-challenged mice are shown. Scale bar=1 mm (left), 100 μm (right). Data were from at least two independent experiments with three to four mice per group. Error bars indicate mean ± standard error of the mean. ***, $p<0.001$.

When the immunological pattern of local inflammation was examined, the expression of *Il17a* as well as *Ifng* and *Il4* was observed in the footpad skin of the *C. albicans*-DTH model. *Candida albicans* sensitization led to an increased level of *Ifng* and *Il4* at day 7. Importantly, the mice showed a marked increase in *Il17a* expression only after *C. albicans* challenge, but no increase in *Il17a* 7 days after *C. albicans* sensitization (Figure 5). These results suggest the presence of a *C. albicans*-specific IL-17 DTH response in the *C. albicans*-DTH model and this model could be suitable for investigating antigen-specific Th17/IL-17 immunity.

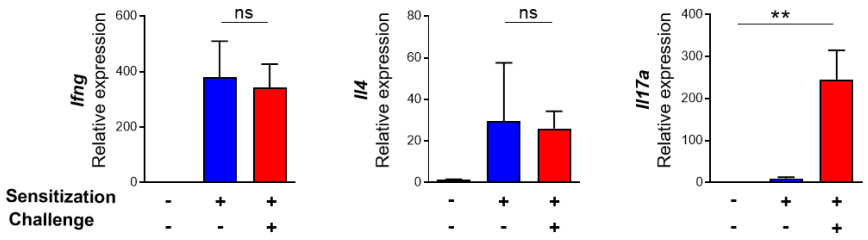

Figure 5. Antigen-specific IL-17 DTH response following *C. albicans* challenge. mRNA expression levels of *Ifng, Il4, and Il17* in the lesional footpad skin were analyzed by qPCR 7 days after *C. albicans* sensitization or 24 hr after *C. albicans* challenge on day 8. Data were from at least two independent experiments with three to four mice per group. Error bars indicate mean ± standard error of the mean. ***, $p<0.001$; ns, not significant.

5. Expression of Peli1 was observed in the footpad skin at steady state

In mouse skin, the expression level of Peli1 at steady state and how it changed in *C. albicans*-DTH model was evaluated. At the steady state, *Peli1* was more highly expressed than other Peli family members, *Peli2* or *Peli3* in the footpad skin (Figure 6A). And, only the expression of *Peli1*, but not *Peli2* or *Peli3*, was significantly reduced although it showed the highest expression following *C. albicans* challenge (Figure 6B). These results suggest that Peli1 was abundantly present in the steady state and that Peli1 might be involved in immunopathogenesis in the *C. albicans*-DTH model.

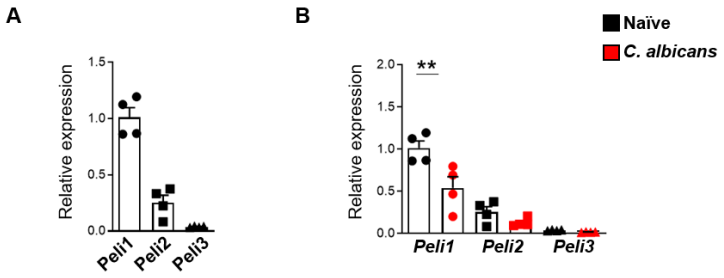

Figure 6. Expression of Peli1, Peli2, and Peli3 in mouse footpad skin. (A) At steady state, mRNA expression levels of *Peli1*, *Peli2,* and *Peli3,* in the footpad skin of mice were analyzed by qPCR. (B) Changes in mRNA expression of *Peli* family members in lesional footpad skin following *C. albicans* challenge are shown. Data were from at least two independent experiments with two to three mice per group Error bars indicate mean ± standard error of the mean. **, $p<0.005$; ns, not significant; Peli1, Pellino-1; Peli2, Pellino-2; Peli3, Pellino-3.

6. Peli1 deficiency led to increased numbers of IL-17-producing CD4$^+$ T cells in popliteal LNs *in vivo*

To determine the effect of Peli1 on *C. albicans*-induced IL-17 immunity *in vivo*, systemic Peli1 KO mouse was generated (Figure 7A). Depletion of *Peli1* in the back skin was confirmed by qPCR (Figure 7B).

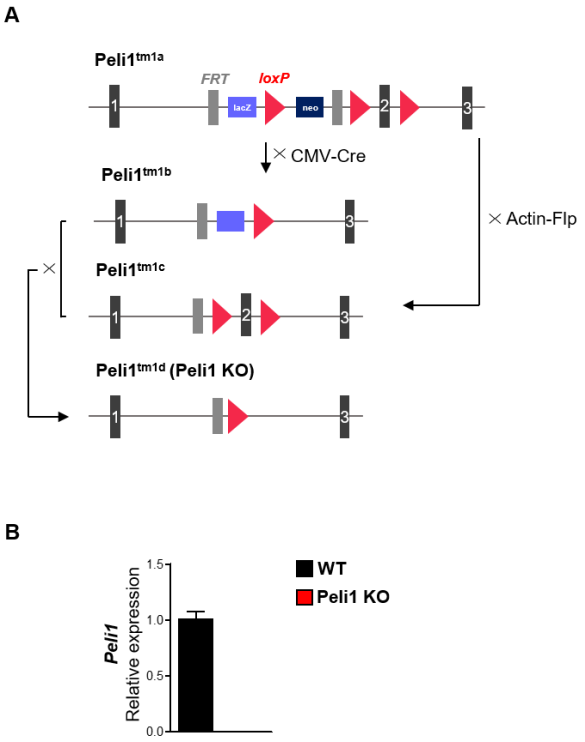

Figure 7. Schematic diagram depicting Peli1 KO mouse generation. (A) Mouse carrying the targeted *Peli1* allele was bred with CMV-Cre mouse to remove the NeoR cassette. Mouse with the *loxP*-flanked *Peli1* allele (*Peli1*$^{fl/fl}$, *Peli1*^{tm1c}) was generated by crossing *Peli1*^{tm1a} mice with ACT-FLPe mouse. *Peli1*^{tm1b} were bred to *Peli1*^{tm1c} to generate systemic Peli1 KO mouse, *Peli1*^{tm1d}. (B) The mRNA levels of *Peli1* in WT and Peli1 KO mice were confirmed by qPCR in back skin. Error bars indicate mean ± standard error of the mean. Peli1, Pellino-1.

To determine whether Peli1-deficiency influences IL-17A production from $CD4^+$ cells, we compared the IL-17A production from popliteal LNs in WT and Peli1 KO mice during *C. albicans* sensitization. $CD4^+$ T cells (Figure 8A, $Live^+CD3e^+TCR\beta^+$ gated), from Peli1 KO mice produced markedly higher IL-17A than those from WT mice, whose IL-17A production was increased compared with that of naïve mice (Figure 8B, C). These findings suggest that Peli1 might play a suppressive role in Th17 cell priming during *C. albicans* sensitization.

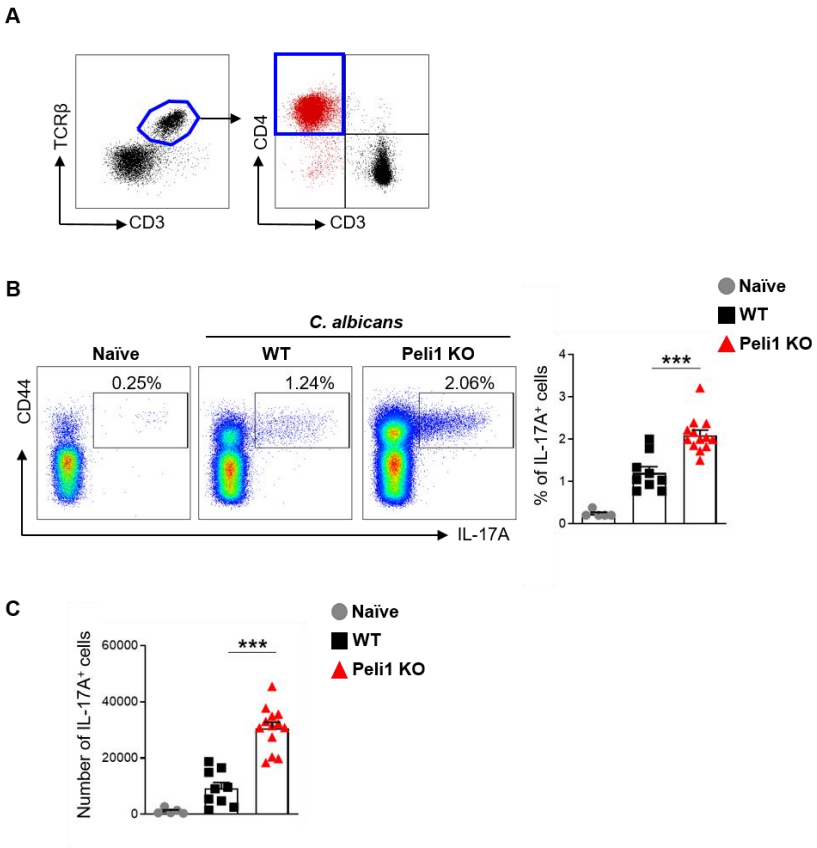

Figure 8. Enhanced Th17 response in popliteal LNs of Peli1 KO mice after *C. albicans* sensitization. (A) The popliteal LNs of naïve, WT, and Peli1 KO mice were analyzed by flow cytometry on day 7 after *C. albicans* sensitization. Gating strategies for $CD4^+$ T cells ($Live^+CD3e^+TCR\beta^+CD4^+$ gated) are shown. (B) Population frequencies of $CD44^+IL\text{-}17A^+$ cells among $CD4^+$ T cells were assessed and the frequencies among $IL\text{-}17^+$ cells of $CD4^+$ T cells are shown. (C) The absolute numbers of $IL\text{-}17^+$ $CD44^+$ cells among $CD4^+$ T cells in naïve, WT, and Peli1 KO mice are shown. Data were from at least two independent

experiments with four to five mice per group. Error bars indicate mean ± standard error of the mean. ***, $p<0.001$; Peli1, Pellino-1.

7. Severe inflammation was developed in the footpad of Peli1 KO mice after *C. albicans* challenge

To examine the changes in the DTH response caused by increased IL-17-producing-$CD4^+$ T cells during *C. albicans* sensitization in Peli1 KO mice, local inflammation was investigated 24 hr after *C. albicans* challenge (Figure 9A). Quantitative analysis of the hematoxylin and eosin images of the footpad skin showed that Peli1 KO mice had greater numbers of infiltrated inflammatory cells (Figure 9B) and increased epidermal thickness (Figure 9C). These results indicate that Peli1 deficiency drives the severe inflammatory phenotype, along with enhanced Th17 cell differentiation in the *C. albicans*-DTH model, and implicate that Peli1-dependent regulatory role for Th17 immunity induced by *C. albicans*.

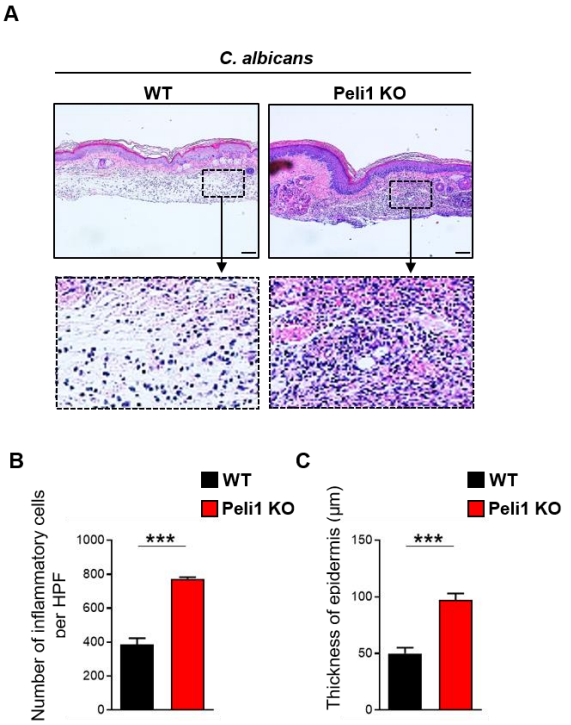

Figure 9. Increased severity of inflammation in the footpad skin of Peli1 KO mice after *C. albicans* challenge. (A) Representative hematoxylin and eosin images of lesional footpad skin showing inflammatory cell infiltration in WT and Peli1 KO mice 24 hr after *C. albicans* challenge are shown. Scale bar=100 μm. (B) The number of infiltrated inflammatory cells in two random HPF was counted using Image J. (C) Thickness of epidermis was measured using Image J from three random epidermis. Data were from at least two independent experiments with two to three mice per group. Error bars indicate mean ± standard error of the mean. ***, $p<0.001$; Peli1, Pellino-1; HPF, high-power field.

8. Specific DC populations were depleted by treatment of mice with diphtheria toxin

Next, because DCs are important in initiating Th17 response, the DC subsets involved in *C. albicans*-induced Th17/IL-17 pathogenesis were determined using DT-induced DC depleted mice (Figure 10A). Steady state skin of WT mouse contains LCs in the epidermis ($CD45^+CD11c^+MHCII^+$ gated) and cDC1 and cDC2 in the dermis ($CD45^+CD11c^+MHCII^+CD64^-$ gated, Figure 10B). DT administration led to the ablation of cDC1 in the dermis and LCs in the epidermis in Langerin-DTR mouse (Figure 10C). In CD301b-DTR mouse, only the $CD301b^+$ dermal DCs, were ablated from the cDC2 population (Figure 10D).

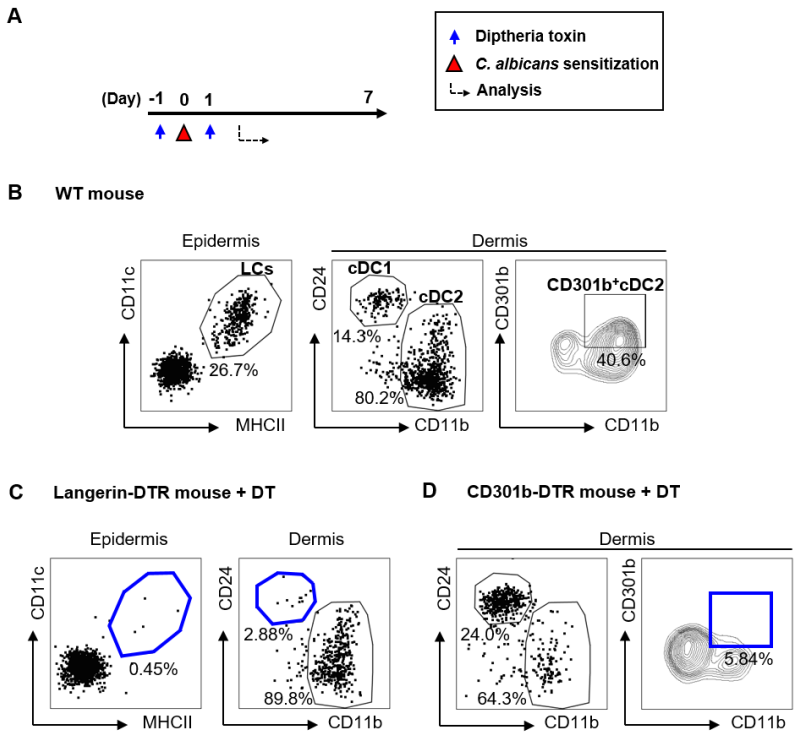

Figure 10. Diphtheria toxin-induced depletion of specific DC subsets in mice. (A) Treatment protocol for DT-induced DC depletion: Langerin-DTR and CD301b-DTR mice were treated intraperitoneally with 1 μg DT 1 day before and 1 day after *C. albicans* sensitization for depletion of specific DC subsets (CD301b$^+$ or Langerin$^+$ DCs). (B) DC subsets in the epidermis (LCs, CD45$^+$CD11c$^+$MHCII$^+$) and in the dermis (cDC1 and cDC2, CD45$^+$CD11c$^+$MHCII$^+$CD64$^-$ gated) of WT mouse were analyzed by flow cytometry in the skin at steady state. (C) Depletion of LCs in the epidermis and cDC1 in the dermis of Langerin-DTR mouse and (D) depletion of CD301b$^+$ DCs from the cDC2 population in the dermis of CD301b-DTR mouse was confirmed by flow cytometry 24 hr after DT treatment.

9. Langerin$^+$ DCs were not involved in mediating Th17 response induced by *C. albicans in vivo*

Upon Langerin$^+$ DC (LCs and cDC1) depletion, WT and DT-treated Langerin-DTR mice had equal ratios of CD4$^+$ T cells to CD8$^+$ T cells, which *C. albicans* sensitization increased significantly (Figure 11A). Additionally, IL-17A expression in CD4$^+$ T cells was comparable with that seen in WT mice after *C. albicans* sensitization (Figure 11B, C). These results indicate that Langerin$^+$ DCs are not necessary in *C. albicans*-induced Th17/IL-17 immunity.

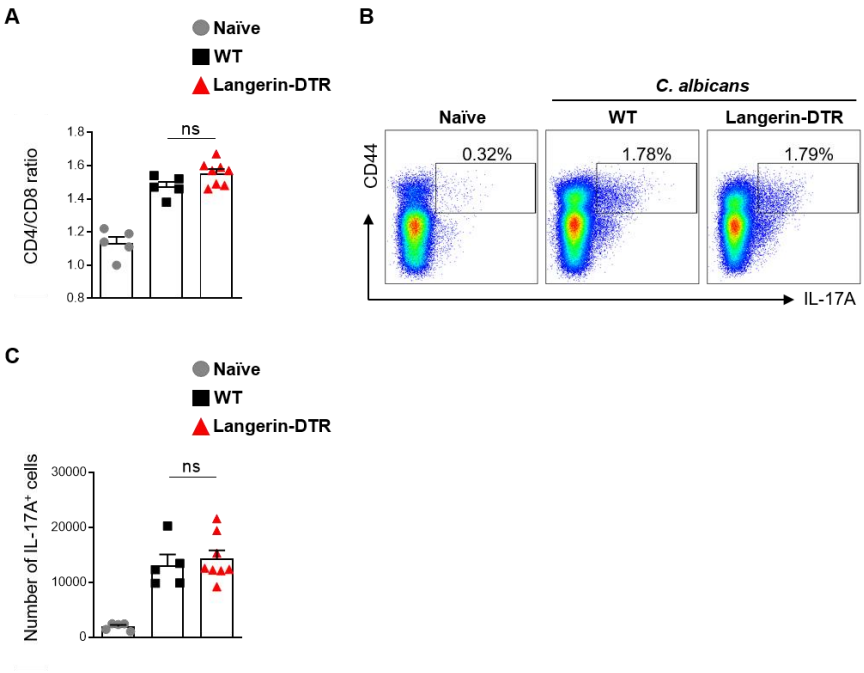

Figure 11. Dispensable role of Langerin$^+$ DCs in *C. albicans*-induced Th17 response *in vivo*. (A) The popliteal LN cells of naïve, WT, and DT-treated Langerin-DTR mice were analyzed by flow cytometry 7 days after *C. albicans* sensitization. The ratios of CD4$^+$ to CD8$^+$ T cells were assessed by flow cytometry. (B) Population frequencies of intracellular IL-17A among CD4$^+$ T cells are shown. (C) The absolute numbers of IL-17$^+$CD44$^+$CD4$^+$ T cells in naïve, WT and Langerin-DTR mice are shown. Data were from at least two independent experiments with two to three mice per group. Error bars indicate mean ± standard error of the mean. ns, not significant.

10. CD301b$^+$ dermal DCs drove Th17 immunity in the sensitization phase of *C. albicans*-DTH model *in vivo*

Next, when CD301b$^+$ dermal DCs were depleted, the ratio of CD4$^+$ to CD8$^+$ T cells decreased as much as that of naïve mice after *C. albicans* sensitization (Figure 12A). Along with the relative decline of CD4$^+$ T cells, depletion of CD301b$^+$ dermal DCs led to a significant decrease in IL-17A expression of CD4$^+$ T cells by half compared with that of WT mice (Figure 12B, C). These findings demonstrate that CD301b$^+$ dermal DCs drive *C. albicans*-induced Th17/IL-17 immunity during the priming of CD4$^+$ T cells.

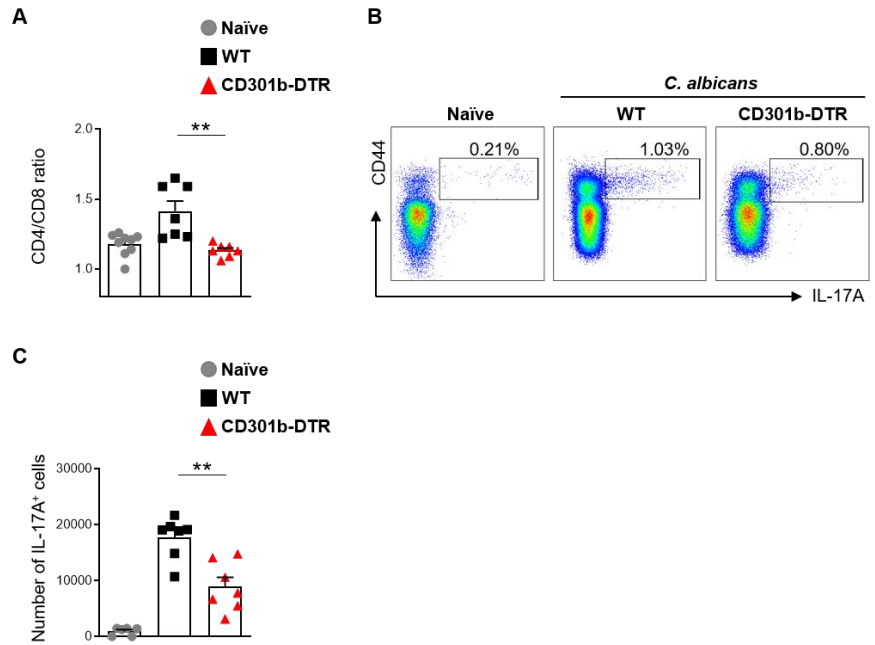

Figure 12. Attenuated Th17 immunity induced by *C. albicans* sensitization after depletion of CD301b$^+$ dermal DCs *in vivo*. (A) The popliteal LNs of naïve, WT, and DT-treated CD301b-DTR mice were analyzed by flow cytometry 7 days after *C. albicans* sensitization. The ratios of CD4$^+$ to CD8$^+$ T cells were assessed by flow cytometry. (B) Population frequencies of CD44$^+$IL-17$^+$ cells among CD4$^+$ T cells are shown. (C) The absolute numbers of CD44$^+$IL-17$^+$ cells of CD4$^+$ T cells in naïve, WT, and CD301b-DTR mice are shown. Data were from at least two independent experiments with two to three mice per group. Error bars indicate mean ± standard error of the mean. $^{**}p<0.005$.

11. DC-specific Peli1 deficiency resulted in elevated IL-17-production of $CD4^+$ T cells from popliteal LNs *in vivo*

Systemic deficiency of Peli1 increased the Th17/IL-17-mediated immunity to *C. albicans*, therefore, the effect of Peli1-deficient DCs in *C. albicans*-induced Th17/IL-17 response was investigated using CD11c-cKO mouse (Figure 13A). Peli1 was confirmed to be depleted in DCs by performing qPCR on purified $CD11c^+$ BMDCs (Figure 13B). $CD4^+$ T cells were significantly higher in CD11c-cKO mice than in WT mice, after *C. albicans* sensitization (Figure 13C). Additionally, IL-17A expression significantly increased in $CD4^+$ T cells (Figure 13D–E). These findings indicate that Peli1 plays a critical role in DCs by regulating the differentiation of IL-17-producing $CD4^+$ T cells.

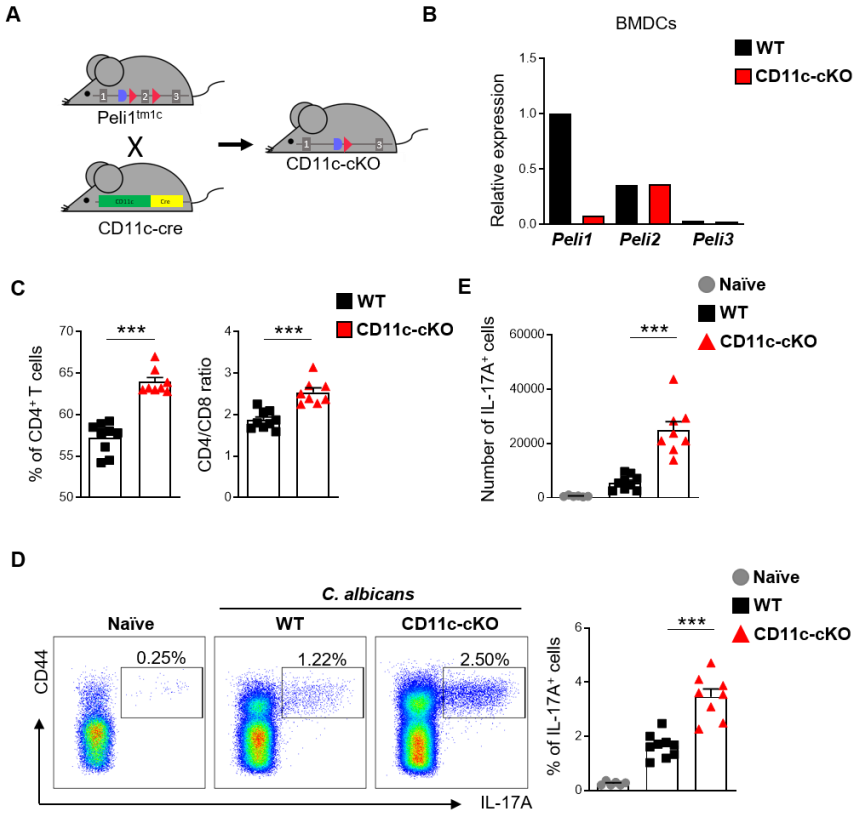

Figure 13. Elevated Th17/IL-17 response in CD11c-cKO mice caused by *C. albicans* sensitization. (A) To generate DC-specific Peli1-deficient mouse, mouse with *loxP*-flanked *Peli1* allele ($Peli1^{fl/fl}$, $Peli1^{tm1c}$) was bred to CD11c-Cre mouse. (B) *Peli1* was determined to have been deleted from DC by conducting qPCR on the $CD11c^+$ BMDCs of CD11c-cKO mouse. (C) The ratios of $CD4^+$ to $CD8^+$ T cells and frequencies of $CD4^+$ T cells are shown. (D) Population frequencies of intracellular IL-17A cells among $CD44^+CD4^+$ T cells in popliteal LNs after *C. albicans* sensitization were examined in naïve, WT, and CD11c-cKO mice. The frequencies of IL-17A of $CD4^+$ T cells are shown. (E)

The absolute numbers of IL-17A of $CD44^{+}CD4^{+}$ T cells in naïve, WT and CD11c-cKO mice are shown. Data were from at least two independent experiments with two to four mice per group. Error bars indicate mean ± standard error of the mean. ***, $p<0.001$.

12. *C. albicans*-induced Th17 response was elevated in the footpad of CD11c-cKO mice

After CD11c-cKO mice received the *C. albicans* challenge, hematoxylin and eosin images of the footpad skin showed that CD11c-cKO mice experienced greater inflammation than did WT mice, as measured by epidermal thickness and inflammatory cellular infiltration (Figure 14A–C). Interestingly, qPCR analysis of *Il17a*, *Ifng* and *Il4* showed that *C. albicans*-induced *Il17a* level selectively increased in CD11c-cKO mice. However, there were no significant differences in *Ifng* and *Il4* levels between WT and CD11c-cKO mice (Figure 14D). These results implicate that Peli1 in DCs is involved in *C. albicans* pathogenesis regulation and that plays a characteristic role only in *C. albicans*-induced specific IL-17 immunity, not Th1- or Th2-associated immune response.

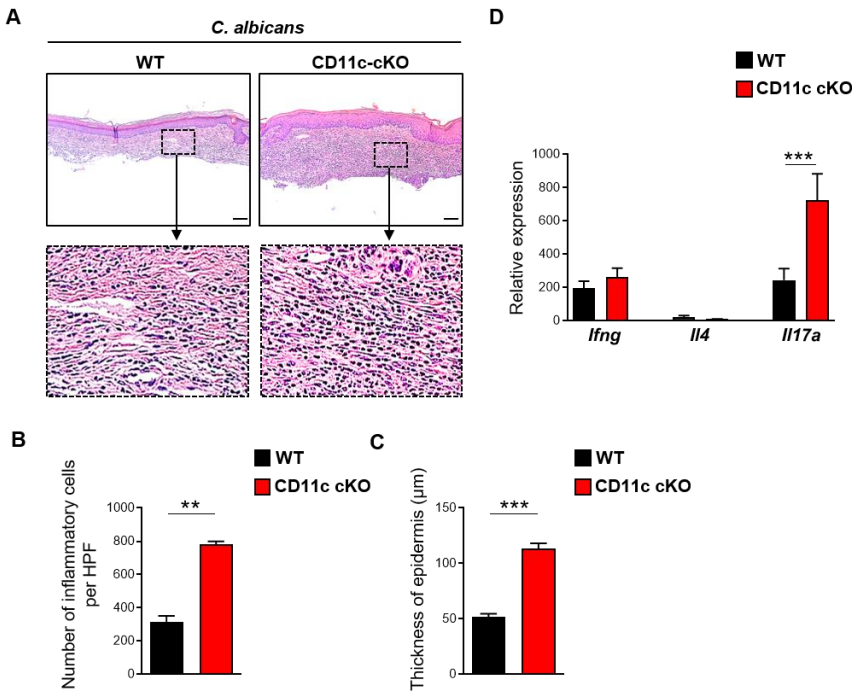

Figure 14. Intensified inflammation in the footpad skin of CD11c-cKO mice caused by *C. albicans* challenge. (A) Hematoxylin and eosin images of the footpad skin 24 hr after *C. albicans* challenge are shown. Scale bar=100 μm. (B) Degree of inflammatory response measured by inflammatory cell infiltration and (C) epidermal thickness, both of which were assessed using Image J. (D) mRNA expression levels of *Ifng*, *Il4* and *Il17a* were analyzed by qPCR of the lesional footpad skin of WT and CD11c-cKO mice. Data were from at least two independent experiments with two to three mice per group. Error bars indicate mean ± standard error of the mean. **, $p<0.05$; ***, $p<0.001$.

13. LC-specific Peli1 deficiency resulted in comparable Th17 immunity in popliteal LNs compared with WT mice *in vivo*

Because Peli1 deficiency in DCs amplified the Th17/IL-17 immune response, experiments were performed to explore whether Peli1 deficiency within LCs is responsible for enhanced Th17 immunity. To this end, huLang-cKO mouse was generated (Figure 15A). The increased IL-17A in $CD4^+$ T cells after *C. albicans* sensitization was unchanged in huLang-cKO mice, indicating that Peli1 deficiency in LCs does not contribute to the increase in Th17 immune response to *C. albicans* (Figure 15B, C). Collectively, these findings demonstrate that Peli1 in DCs except LCs, is critical to the pathogenesis of the *C. albicans*-DTH model via negative regulation of Th17/IL-17 immunity.

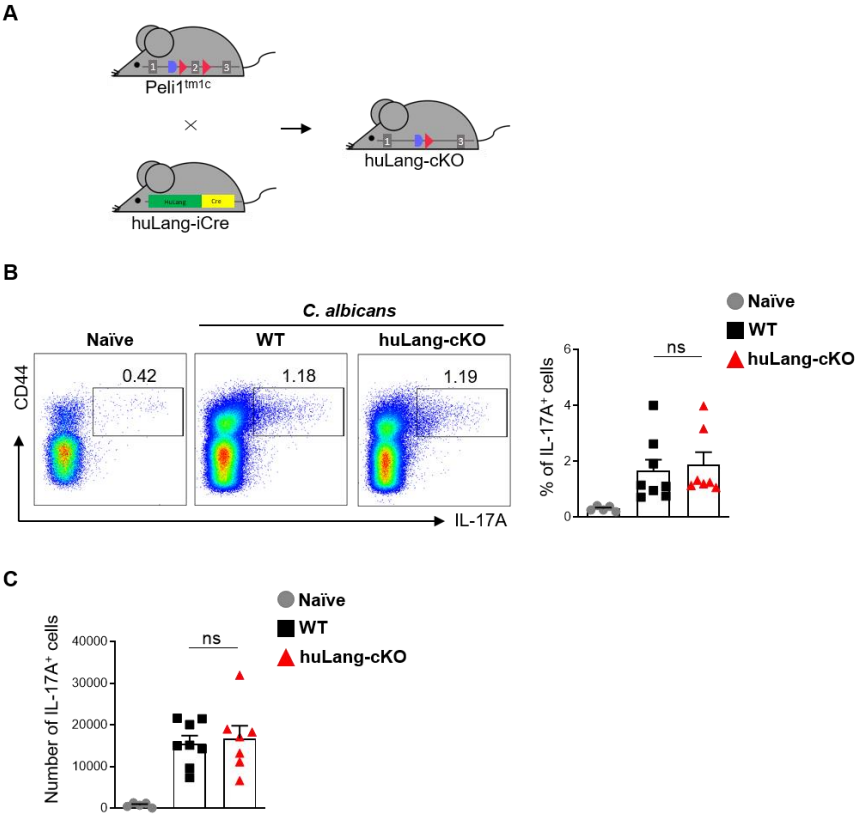

Figure 15. Comparable Th17 response in popliteal LNs of WT and huLang-cKO mice after *C. albicans* sensitization. (A) To generate huLang-cKO mouse, mouse with the *loxP*-flanked *Peli1* allele ($Peli1^{fl/fl}$, $Peli1^{tm1c}$) was bred with huLang-Cre mouse. (B) After WT and huLang-cKO mice were sensitized with *C. albicans*, intracellular $CD44^{+}IL\text{-}17A^{+}$ expression in $CD4^{+}$ T cells from popliteal LNs was analyzed after 7 days using flow cytometry. Population frequencies of $CD44^{+}IL\text{-}17A^{+}$ cells are shown ($Live^{+}CD3e^{+}TCR\beta^{+}CD4^{+}$ gated). The frequencies of $IL\text{-}17A^{+}$ of $CD4^{+}$ T cells

are shown. (C) Absolute numbers of $CD44^{+}IL\text{-}17A^{+}$ cells in naïve, WT, and huLang-cKO mice were analyzed. Data were from at least two independent experiments with two to four mice per group. Error bars indicate mean ± standard error of the mean. ns, not significant.

14. Peli1-deficient BMDCs augmented TGF-β and IL-1β in response to HK *C. albicans in vitro*

Results to date with *in vivo* experiments demonstrate that Peli1 in dermal DCs is an important part of regulating the *C. albicans*-specific IL-17 DTH response. Thus, to identify how the Peli1 in dermal DCs controls Th17 immunity, the ability of DCs to prime T cells was investigated using BMDCs of WT and CD11c-cKO mice (Figure 16A, B). Analysis of the DC surface molecules showed that the expression of MHCII and CD80, which increased by HK *C. albicans*, were not different considerably with or without the presence of Peli1 24 hr after HK *C. albicans* stimulation (Figure 16C).

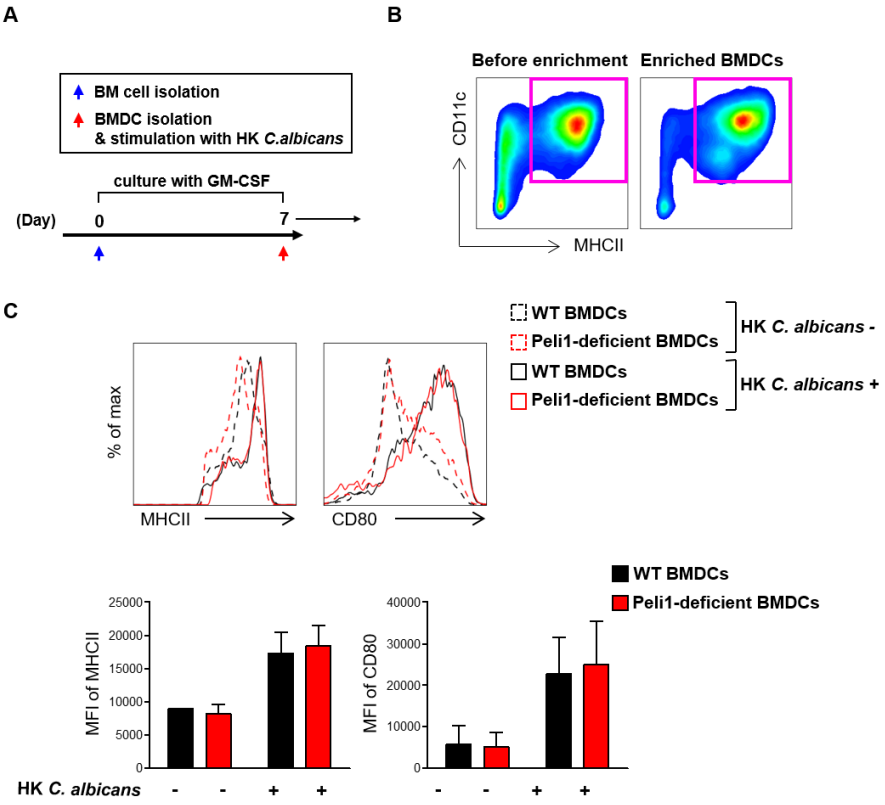

Figure 16. Similar expression in surface molecules between WT and Peli1-deficient BMDCs. (A) BM cells of WT and CD11c-cKO mice were cultured with GM-CSF for 7 days. The purified BMDCs were then stimulated with HK *C. albicans* for the indicated times. (B) Before and after enrichment of BMDCs were analyzed by flow cytometry. (C) Representative histograms showing expression levels of MHCII and CD80 24 hr after the stimulation in WT and Peli1-deficient BMDCs, were analyzed by flow cytometry. MFI (mean fluorescent intensity) of MHCII and CD80 are also shown. Data were from at least two independent experiments. Error bars indicate mean ± standard error of the mean. HK; heat-killed.

TGF-β, IL-6, IL-1β and IL-23 produced from DCs are critical for the differentiation of naïve T cells into Th17. It was noted that HK *C. albicans* markedly increased IL-6 and IL-1β mRNA and protein in BMDCs *in vitro*. The expression of *Tgfb* and *Il1b* in the early stages of stimulation was slightly higher in Peli1-deficient BMDCs; *Tgfb* and *Il1b* levels were significantly higher, in particular, 1 hr after the stimulation (Figure 17A). Consistent with their corresponding mRNA levels, protein levels of IL-6 and TGF-β were significantly higher in Peli1-deficient BMDCs (Figure 17B). Taken together, these results indicate that Peli1 in dermal DCs may regulate Th17 immunity to *C. albicans* by controlling cytokine production of DC during the differentiation of naïve T cells into Th17 cells.

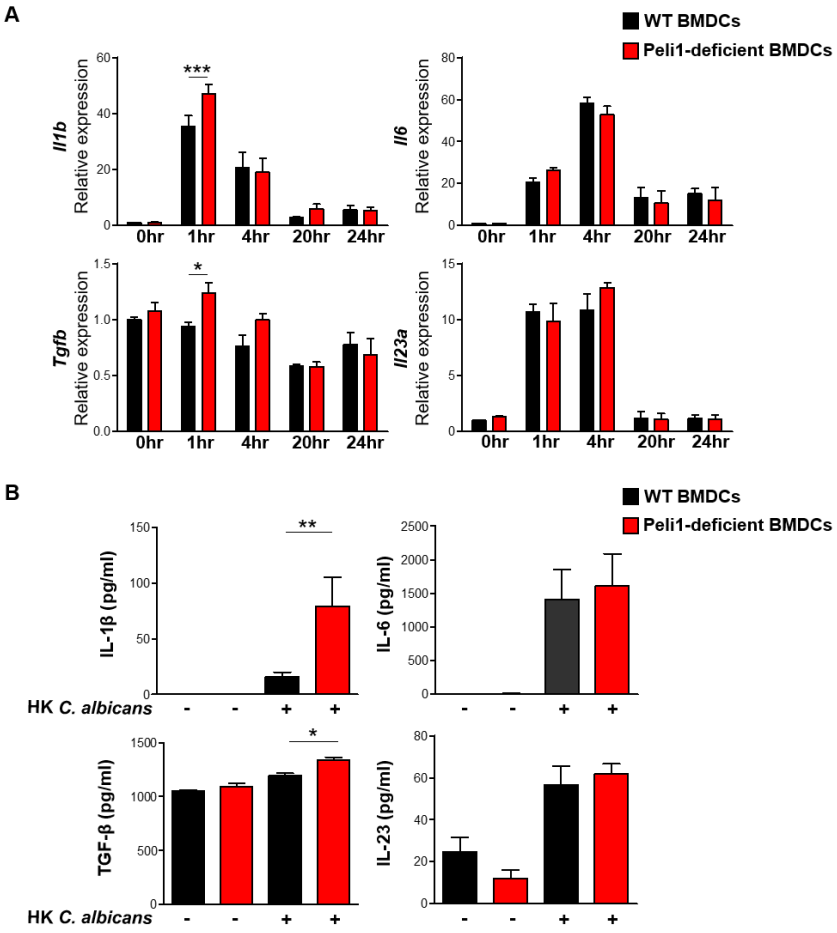

Figure 17. Increased cytokine levels in Peli1-deficient BMDCs *in vitro*. (A) After *in vitro* stimulation with HK *C. albicans*, the expression levels of *Il1b, Il6, Tgfb* and *Il23* mRNA in the BMDCs of WT and CD11c-cKO mice were analyzed by qPCR at the indicated times. (B) IL-1β, IL-6, TGF-β and IL-23 protein levels were analyzed by ELISA 24 hr after the stimulation. Data were from at least three independent experiments. Error bars indicate mean ± standard error of the mean. *, $p<0.05$; **, $p<0.005$; ***, $p<0.001$; HK, heat-killed.

IV. DISCUSSION

From among the E3 ubiquitin ligases, members of Peli family have been identified as critical moderators of diverse immune responses[18-20]. Since the role of Peli1 in DCs under cell-specific contexts has not been the subject of investigations to date, this study is, to our knowledge, the first to determine that Peli1 in DCs plays a crucial role in modulating Th17/IL-17 immune response using a *C. albicans*-DTH model.

IL-17 immunity is commonly involved in different infection models of *C. albicans*, including cutaneous, mucosal, and, oral candidiasis[22,23]. Of the IL-17-producing cells including innate lymphoid cells, γδ T cells, neutrophils, and Th17 cells, Th17 cells are important for adaptive immune system to *C. albicans*[22,24]. Distinctly, Th17 cells provide protection against secondary cutaneous infection, whereas Th1 cells protect against secondary systemic infections[22,25]. In this study, *Ifng* and *Il17a* levels were considerably increased in the *C. albicans*-DTH model. Although gene expression of *Ifng* was already increased during the sensitization phase, *Il17a* was markedly increased only after challenge with antigen, indicating that IL-17 immune response is a *C. albicans*-specific DTH response. Although the DTH model is suitable to study T cell-mediated immune responses, the cellular and molecular regulation of IL-17-mediated DTH response has been poorly understood yet.

Peli1-deficient mouse had a stronger Th17/IL-17 response than WT mouse, suggesting a Peli1-dependent regulation of Th17 immunity induced by *C. albicans*. These results were consistent with those of previous studies showing a

negative regulatory role for Peli1 in T cell activation. Additionally, Peli1 had a protective role of systemic lupus erythematosus through inhibiting noncanonical nuclear factor-κB activation and B cell antibody production[20].

In DC-specific Peli1-deficient mouse, *C. albicans*-induced Th17 response, not Th1-associated response, was markedly exaggerated. This increase in Th17 response was similar to the observed results when Peli1 was systemically depleted. These findings indicate that Peli1 in DCs crucially functions to regulate Th17 DTH response in this model and highlight the importance of studying the cell-specific role of Peli1 in future.

Th17 response to *C. albicans* in DT-treated Langerin-DTR mouse, depleted all Langerin$^+$ DCs, including LCs and Langerin$^+$ cDC1, was comparable to WT mouse. These findings are somewhat inconsistent with those of the previous studies demonstrating that Th17 response to the epicutaneous *C. albicans* infection is mainly mediated by LCs while CD103$^+$ dermal DCs are required for differentiation of Th1 cells[22,25]. To mediate antigen-specifc Th17 cell priming, LCs produce IL-6, which is required for Th17 cell differentiation, by interacting with *C. albicans* yeast in a dectin-1-dependent manner. During the epicutaneous infection of the yeast form of *C. albicans, C. albicans* yeast undergoes morphological changes to the pseudo-hyphae form during dermal invasion, making it impossible to dectin-1 ligation[25]. Meanwhile, it was reported that LCs are not involved in intradermal injection of *C. albicans* infection models that largely bypass the interaction with epidermal LCs[26]. Rather, our study showed that depletion of CD301b$^+$ dermal DCs in DT-treated CD301b-DTR mouse, significantly attenuated Th17 response to *C. albicans*. An involvement of CD301b$^+$ dermal DCs is not much appreciated in Th17 response during *C.*

albicans epicutaneous application and dermal injection models. However, the difference of induction method between epicutaneous application and intradermal injection *C. albicans* infection models may explain the crucial role of CD301b$^+$ dermal DCs to induce Th17 immunity in *C. albicans*-DTH model[25].

Notably, it has been reported that cDC2 including CD301b$^+$ DCs also expresses dectin-1 which would sense and initiate innate immunity to *C. albicans*[25,27]. Therefore, CD301b$^+$ dermal DCs could facilitate dectin-1-dependent Th17 response to *C. albicans* yeast exposed via dermal or subcutaneous injection route. Besides, CD301b$^+$ dermal DCs induce IL-17 production from γδ T cells through cutaneous sensory nerves-derived calcitonin gene-related peptide which stimulates CD301b$^+$ dermal DCs to produce IL-23[28]. It has also been reported that CD301b$^+$ dermal DCs are involved in Th17 cell differentiation in response to pyogenic streptococcal infection in the nasal mucosa[29]. In line with these results, CD301b$^+$ dermal DCs have the capacity to induce Th17 reaction in a dectin-1-dependent manner under specific conditions.

It is well-documented that the combined actions of IL-23, IL-6, IL-1β, and TGF-β are required for Th17 cell differentiation[25]. Still, the importance of individual cytokines in generating Th17 cells remains controversial. Many studies reported that both IL-1β and IL-6 are required for Th17 cell differentiation in peripheral tissues including skin[30]. In particular, recent studies have highlighted a critical role for IL-1/IL-1 receptor signaling in early Th17 cell differentiation was reported[30-32]. Consistent with these findings, we observed that *in vitro* stimulation with HK *C. albicans* rapidly increased IL-1β and IL-6 with a slight increase of TGF-β and IL-23 from BMDCs. Interestingly, the level of IL-1β and TGF-β were much highly increased in Peli1-deficient

BMDCs compared to WT BMDCs by HK *C. albicans* stimulation. Therefore, it is possible that Peli1 in DCs regulates Th17 cell differentiation by modulating the expression of IL-1β and TGF-β in DCs.

Many Th17/IL-17 immune-mediated diseases are known to be associated with the deregulation of ubiquitin signaling; these diseases include psoriasis, cancer, inflammatory bowel disease, rheumatoid arthritis, and asthma[8,9]. Indeed, recent reports suggested that Peli1 is important for inducing psoriatic inflammation by activating the hyperproliferation of keratinocytes[33]. In addition, Peli1 has been reported to be strongly related to carcinogenesis[34]. Moreover, dysregulation in the nuclear factor-κB pathway was typically observed in inflammatory bowel disease including Crohn's disease and ulcerative colitis, and Peli1 activates nuclear factor-κB pathways[35]. A gastrointestinal tract-targeting Peli1 inhibitor for the treatment of ulcerative colitis is now under development (Bridge Biotherapeutics, Seongnam, Korea).

However, in the view of that Peli1 has not been studied in *C. albicans*-induced Th17 immunity, this study suggests that Peli1 would be a promising therapeutic target for controlling the response to *C. albicans* infection via regulation of Th17/IL-17 immunity. Moreover, we identified that Peli1 in DCs regulates Th17 cell priming in the deep dermal *C. albicans* infection, which implies that Peli1 in DCs could be targeted for the management of deep dermal *C. albicans* infection.

V. CONCLUSION

This study highlights the crucial role of Peli1, an E3 ubiquitin ligase, in DCs during *C. albicans*-induced Th17/IL-17 immune reaction. To the best of our knowledge, this is the first study to show how Peli1 within DCs regulates Th17/IL-17 immunity using DC-specific Peli1-deficient mouse. In the present study, the *C. albicans*-DTH model exhibited an increase in Th17 cell counts and a remarkable antigen-specific IL-17 DTH response upon *C. albicans* challenge. Th17 immune response was attenuated in DT-treated CD301b-DTR mouse, demonstrating that $CD301b^+$ dermal DCs are an important part of inducing Th17/IL-17 immunity during the priming of $CD4^+$ T cells following *C. albicans* sensitization. Moreover, DC-specific Peli1-deficiency significantly increased the *C. albicans*-specific Th17/IL-17 DTH response *in vivo*, and Peli1-deficient BMDCs produced higher levels of TGF-β and IL-1β than WT BMDCs after *in vitro* stimulation with HK *C. albicans*. In brief, the results of this study demonstrate that Peli1 in $CD301b^+$ dermal DCs exerts an important role in the regulation of Th17 cell priming via IL-1β- and TGF-β-dependent pathways in *C. albicans*-DTH model. These findings suggest that targeting Peli1 in certain DC subsets is a feasible method for controlling Th17/IL-17 immunity against severe invasive *C. albicans* infection.

REFERENCES

1. Kashem SW, Haniffa M, Kaplan DH. Antigen-Presenting Cells in the Skin. Annu Rev Immunol 2017;35:469 1–499.

2. Honda T, Egawa G, Kabashima K. Antigen presentation and adaptive immune responses in skin. Int Immunol 2019;31:423–429.

3. Adrian E. Morelli, Peter Rubin J, Geza Erdos, Olga A. Tkacheva, Alicia R. Mathers, Alan F. Zahorchak, et al. CD4+ T Cell Responses Elicited by Different Subsets of Human Skin Migratory Dendritic Cells. J Immunol 2005; 175:7905–7915.

4. Drutman SB, Trombetta ES. Dendritic cells continue to capture and present antigens after maturation in vivo. J Immunol 2010;185:2140–2146.

5. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–252.

6. Luckheeram RV, Zhou R, Verma AD, Xia B. CD4+T Cells: Differentiation and Functions. Clin Dev Immunol 2012;2012:925135.

7. Muranski P, Restifo NP. Essentials of Th17 cell commitment and plasticity. Blood 2013;121:2402–2414.

8. Tesmer LA, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. Immunol Rev 2008;223:87–113.

9. Wilke CM, Bishop K, Fox D, Zou W. Deciphering the role of Th17 cells in human disease. Trends Immunol 2011;32:603–611.

10. Fulda S, Rajalingam K, Dikic I. Ubiquitylation in immune disorders and cancer: from molecular mechanisms to therapeutic implications. EMBO Mol Med 2012;4:545–56.

11. Cho JJ, Xu Z, Parthasarathy U, Drashansky TT, Helm EY, Zuniga AN, et al. Hectd3 promotes pathogenic Th17 lineage through Stat3 activation and Malt1 signaling in neuroinflammation. Nat Commun 2019;10:701.

12. Komander D. The emerging complexity of protein ubiquitination. Biochem Soc Trans 2009;37:937–53.

13. Skaar JR, Pagan JK, Pagano M. SCF ubiquitin ligase-targeted therapies. Nat Rev Drug Discov 2014;13:889–903.

14. Aki D,Li Q, Li H, Liu YC, Lee JH. Immune regulation by protein ubiquitination: roles of the E3 ligases VHL and Itch. Protein Cell 2019;10:395–404.

15. Moynagh PN. The roles of Pellino E3 ubiquitin ligases in immunity. Nat Rev Immunol 2014;14:122–131.

16. Wei J, Chang M, Sun SC. Peli: a family of signal-responsive E3 ubiquitin ligases mediating TLR signaling and T-cell tolerance. Cell Mol Immunol 2012;9:113–122.

17.Chang M, Wei J, Sun SC. Peli1 facilitates TRIF-dependent Toll-like receptor signaling and proinflammatory cytokine production. Nat Immunol 2009;10:1089–1095.

18. Chang M, Wei J, Chang JH, Xiao YC, Brittain GC, Yu J, et al. Peli1 negatively regulates T-cell activation and prevents autoimmunity. Nat Immunol 2011;12:1002–1009.

19. Xiao Y, Jin J, Chang M, Chang JH, Hu H, Zhou X, et al. Peli1 promotes microglia-mediated CNS inflammation by regulating Traf3 degradation. Nat Med 2013;19:595–602.

20. Liu J, Huang X, Hao S, Yan W, Liu M, Xu J, et al. Peli1 negatively

regulates noncanonical NF-κB signaling to restrain systemic lupus erythematosus. Nat Commun 2018;9:1136.

21. Kamala T. Hock immunization: A humane alternative to mouse footpad injections. J Immunol Methods. 2007;328:204–214.

22. Kashem SW, Kaplan DH. Skin Immunity to *Candida albicans.* Trends Immunol 2016;37:440–450.

23. Mengesha BG, Conti HR. The Role of IL-17 in Protection against Mucosal *Candida* Infections. J Fungi 2017;3:52.

24. Hernández-Santos N, Gaffen SL. Th17 cells in immunity to *Candida a lbicans.* Cell Host Microbe 2012;11:425–435.

25. Kashem SW, Igyarto BZ, Gerami-Nejad M, Kumamoto Y, Mohammed JA, Jarrett E, et al. *Candida albicans* Morphology and Dendritic Cell Subsets Determine T Helper Cell Differentiation. Immunity 2015;42:356–366.

26. Igyártó BZ, Haley K, Ortner D, Bobr A, Gerami-Nejad M, Edelson BT, et al. Skin-Resident Murine Dendritic Cell Subsets Promote Distinct and Opposing Antigen-Specific T Helper Cell Responses. Immunity 2011;35:260–72.

27. Le HT, Tran VG, Kim W, Kim J, Cho HR, Kwon B. IL-33 Priming Regulates Multiple Steps of the Neutrophil-Mediated Anti-Candida albicans Response by Modulating TLR and Dectin-1 Signals. J Immunol 2012;189:287–95.

28. 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;43:515–526.

29. Linehan JL, Dileepan T, Kashem SW, Kaplan DH. Cleary P, Jenkinsa MK. Generation of Th17 cells in response to intranasal infection requires TGF-β1 from dendritic cells and IL-6 from CD301b+ dendritic cells. Proc Natl Acad Sci USA 2015;112:12782–12787.

30. Hu W, Troutman TD, Edukulla R, Pasare C. Priming Microenviron ments Dictate Cytokine Requirements for T Helper 17 Cell Lineage Co mmitment. Immunity 2011;35:1010–1022.

31. Ikeda S, Saijo S, Murayama MA, Shimizu K, Akitsu A, Iwakura Y. Excess IL-1 Signaling Enhances the Development of Th17 Cells by Do wnregulating TGF-β-Induced Foxp3 Expression. J Immunol 2014;192:144 9–1458.

32. Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang H S, et al. Critical regulation of early Th17 cell differentiation by IL-1 sign aling. Immunity 2009;30:576–87.

33. Kim S, Lee SY, Bae S, Lee JK, Hwang K, Go H, et al. Pellino1 promotes chronic inflammatory skin disease via keratinocyte hyperproliferation and induction of the T helper 17 response. Exp Mol Med 2020;52:1537–1549.

34. Jeon YK, Kim CK, Hwang KR, Park HY, Koh J, Chung DH, et al. Pellino-1 promotes lung carcinogenesis via the stabilization of Slug and Snail through K63-mediated polyubiquitination. Cell Death Differ 2017;24:469–480.

35. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017;2:17023.

ABSTRACT

Candida albicans 유발 interleukin-17 면역반응에서 수지상세포의 Pellino-1 의존적인 역할 규명

< 지도교수 이민걸 >

연세대학교 대학원 의과학과

박 제 연

우리 몸은 수많은 종류의 외부항원에 노출되어 있지만 우리의 면역체계는 위협적인 외부항원을 효율적으로 구별하고 적절한 면역반응을 유도함으로써 우리 몸을 보호하고 있다. 이러한 항원 특이적 보호면역반응은 위협 신호를 감지하여 성숙된 수지상세포에 의해 시작된다. 수지상세포에 의해 분화된 Th 세포의 종류 (Th1, Th2, Th17) 중, Interleukin-17 (IL-17)을 특징적으로 분비하는 Th17 세포는 주로 박테리아 및 진균에 대한 숙주방어에 중요한 역할을 한다. 또한, 건선, 류마티스 관절염, 염증성 장염, 천식 등 많은 질병의 발병기전에도 관여하는데 이러한 질병은 유비퀴틴 신호전달의 조절이상과 연관되어 있다고 밝혀졌다. E3 연결효소인 Pellino (Peli)에 속해 있는 Peli1은 다양한 면역세포에서 서로 다른 역할을 수행한다고 보고되었다. 그러나 기존 연구들은 전신 Peli1 넉아웃 마우스를 이용했으므로 세포특이적인 Peli1의 기능을 이해하기에는 한계가 있다. 또한 현재까지 T세포 적응면역에

핵심적인 역할을 하는 수지상세포에서 Peli1이 어떠한 역할을 하는지 알려진바 없다. 이에 본 연구에서는 Th17 대표 피부염 모델인 *Candida albicans* 지연형 과민증 마우스 모델을 이용하여, Th17/IL-17 세포 분화/활성화 과정에서 수지상세포 내 Peli1의 기능을 규명하고자 하였다. 본 연구에서, *C. albicans* 감작에 의해 국소림프절에서 세포내 IL-17을 분비하는 CD4 T 세포의 발현이 증가되었다. 또한 *C. albicans* 재노출에 의한 발바닥 부종, 염증세포의 침윤의 국소 염증반응과 함께 항원 특이적 IL-17 지연형 과민형 반응을 관찰하였다. 이러한 항원 특이적 Th17 반응에서 세포특이적 Peli1 역할에 대해 알아보기 위하여 수지상세포 특이적 Peli1 결핍 마우스에 이 모델을 적용하였다. 염증정도를 야생형과 비교하였을때 수지상세포 특이적 Peli1 넉아웃 마우스에서는 *C. albicans* 유도 Th17/IL-17 반응이 악화되었고 Peli1 결핍 수지상세포에서는 Th17 세포분화에 중요하다고 알려진 사이토카인이 높게 생성 되었다.

결론적으로, Langerin^{+} DC를 제외한 특정 피부 수지상세포 아형의 Peli1이 사이토카인 분비를 조절함으로써 *C. albicans* 지연형 과민증 모델의 IL-17-T 세포 면역반응에 관여할 것으로 예상된다. 향후 침습성 *C. albicans* 감염에 있어서, 수지상세포의 Peli1을 타겟으로 한 새로운 IL-17 의존적 면역반응 조절 물질 개발이 가능하리라 생각된다.

핵심되는 말: 펠리노-1 (Pellino-1), *C. albicans*, 수지상세포, IL-17

PUBLICATION LIST

1. Kim HJ, Kim SH, Park J, Lee M, Kim DS, Lee MG. Up-regulation of receptor antagonist interleukin-1 family members in psoriasis and their regulation by pro-inflammatory cytokines. J Dermatol Sci 2016;82:204–6.
2. Kim HJ, Kim SH, Kim TG, Park JY, Lee M, Kim DS, et al. Interleukin-21 receptor signalling is not critically required for imiquimod-induced psoriasiform dermatitis in mice. Exp Dermatol 2018;27:191–195.
3. Kim TG, Kim SH, Park J, Choi W, Sohn M, Na HY, et al. Skin-Specific CD301b + Dermal Dendritic Cells Drive IL-17-Mediated Psoriasis-Like Immune Response in Mice. J Invest Dermatol 2018;138:844–853.
4. Lee M, Kim SH, Kim TG, Park J, Lee JW, Lee MG. Resident and monocyte-derived Langerhans cells are required for imiquimod-induced psoriasis-like dermatitis model. J Dermatol Sci 2018;91:52–59.
5. Lee HJ, Kim TG, Kim SH, Park JY, Lee M, Lee JW et al. Epidermal Barrier Function Is Impaired in Langerhans Cell-Depleted Mice. J Invest Dermatol 2019;139:1182–1185.
6. Park J, Kim TG, Lee JW, Kim SH, Oh J, Kim SM, et al. Type 2 immunity plays an essential role for murine model of allergic contact dermatitis with mixed type 1/type 2 immunity mimicking human. *(Submitted)*