







ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Fatty acid-binding protein 3 controls contact hypersensitivity through regulating skin dermal V γ 4⁺ γ / δ T cell in a murine model

Shuhei Kobayashi¹  | Hai The Phung²  | Yoshiteru Kagawa¹  | Hirofumi Miyazaki¹  | Yu Takahashi¹ | Atsuko Asao² | Takashi Maruyama³  | Akihiko Yoshimura⁴ | Naoto Ishii²  | Yuji Owada¹

¹Department of Organ Anatomy, Tohoku University Graduate School of Medicine, Sendai, Japan

²Department of Microbiology and Immunology, Tohoku University Graduate School of Medicine, Sendai, Japan

³Mucosal Immunology Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA

⁴Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo, Japan

Correspondence

Shuhei Kobayashi and Yuji Owada, Department of Organ Anatomy, Tohoku University Graduate School of Medicine, Sendai, 980-8575, Japan.
Emails: s_kobayashi@med.tohoku.ac.jp; Owada@med.tohoku.ac.jp

Abstract

Background: Fatty acid-binding protein 3 (FABP3) is a cytosolic carrier protein of polyunsaturated fatty acids (PUFAs) and regulates cellular metabolism. However, the physiological functions of FABP3 in immune cells and how FABP3 regulates inflammatory responses remain unclear.

Methods: Contact hypersensitivity (CHS) induced by 2,4-dinitrofluorobenzene (DNFB) and fluorescein isothiocyanate was applied to the skin wild-type and *Fabp3*^{-/-} mice. Skin inflammation was assessed using FACS, histological, and qPCR analyses. The development of γ / δ T cells was evaluated by a co-culture system with OP9/D11 cells in the presence or absence of transgene of FABP3.

Results: *Fabp3*-deficient mice exhibit a more severe phenotype of contact hypersensitivity (CHS) accompanied by infiltration of IL-17-producing V γ 4⁺ γ / δ T cells that critically control skin inflammation. In *Fabp3*^{-/-} mice, we found a larger proportion of V γ 4⁺ γ / δ T cells in the skin, even though the percentage of total γ / δ T cells did not change at steady state. Similarly, juvenile *Fabp3*^{-/-} mice also contained a higher amount of V γ 4⁺ γ / δ T cells not only in the skin but in the thymus when compared with wild-type mice. Furthermore, thymic double-negative (DN) cells expressed FABP3, and FABP3 negatively regulates the development of V γ 4⁺ γ / δ T cells in the thymus.

Conclusions: These findings suggest that FABP3 functions as a negative regulator of skin inflammation through limiting pathogenic V γ 4⁺ γ / δ T-cell generation in the thymus.

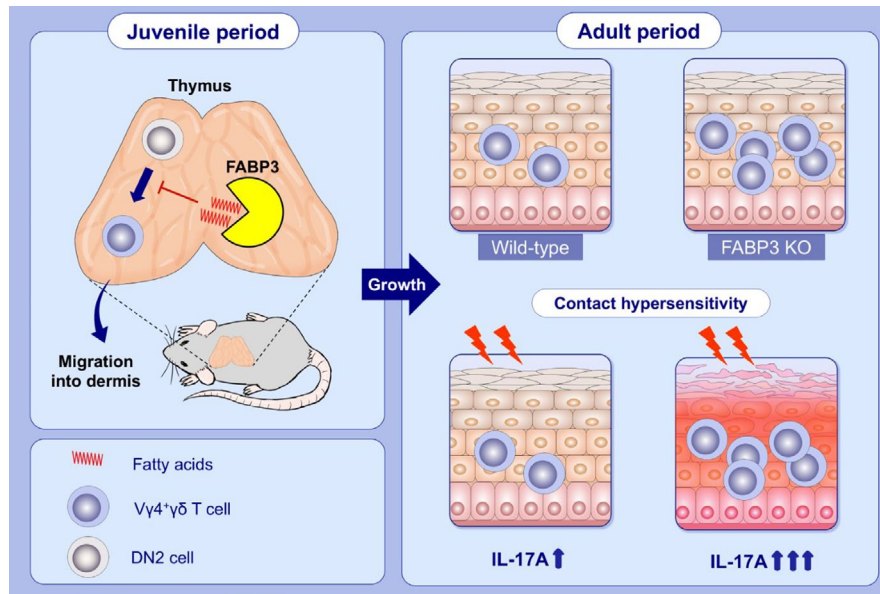
KEYWORDS

contact hypersensitivity, FABP3, skin inflammation, γ / δ T-cell development

Abbreviations: CHS, contact hypersensitivity; DN cell, double-negative cells; DNFB, 2,4-dinitrofluorobenzene; FA, fatty acid; FABP, fatty acid-binding protein; FITC, fluorescein isothiocyanate; LN, lymph node; PUFA, polyunsaturated fatty acid; Tc, cytotoxic T cell; TF, transcription factor; Th, helper T cell.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2020 The Authors. *Allergy* published by European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd



GRAPHICAL ABSTRACT

FABP3 negatively regulates the differentiation of thymic DN2 cells into $V\gamma 4^+$ γ/δ T cells. FABP3-deficient mice exhibit higher frequency of $V\gamma 4^+$ γ/δ T cells in skin of both juvenile and adults. FABP3 deficiency in $V\gamma 4^+$ γ/δ T cells enhances production of high amounts of IL-17A, which contributes to the pathogenesis of contact hypersensitivity. DN2, double negative cell; FABP3, fatty acid-binding protein 3; KO, knockout.

Abbreviations: DN2, double negative cell; FABP3, fatty acid-binding protein 3; KO, knockout.

1 | INTRODUCTION

The skin harbors various immune cells¹ and function as a frontier barrier that protects the host from external threats. γ/δ T cells express TCR γ/δ , but not TCR α/β , and these cells are divided into some subsets including $V\gamma 5$ or $V\gamma 4$ expressing γ/δ T cells ($V\gamma 5^+$ γ/δ T cells and $V\gamma 4^+$ γ/δ T cells, respectively).² Although γ/δ T cells are a small population among circulating T cells, some certain subsets of γ/δ T cells are more presented in epithelial tissues, such as skin and intestine.³ The skin is divided into two major compartments: the epidermis and the dermis. In mice, while epidermal compartment predominantly contains $V\gamma 5^+$ γ/δ T cells, $V\gamma 4^+$ γ/δ T cells abundantly reside in dermal compartment.⁴ More than half of $V\gamma 4^+$ γ/δ T cells are IL-17-producing cells, and these cells are a major source of IL-17 production in the early stages of skin inflammation.⁵ It has been shown that γ/δ T cells promote inflammation and insulin resistance during high-fat diet-induced obesity⁶ and are involved in the pathology of psoriatic dermatitis.⁷ However, it remains elusive how systemic and/or cellular lipid metabolism affects the activation of γ/δ T cells and consequently the pathology of skin inflammation.

T cell precursors go through the positive and negative selection in the thymus and differentiate into $CD4^+$, $CD8^+$, and γ/δ T cells. These precursors display a $CD4^-CD8^-$ (double negative, DN) phenotype and are further classified into $CD44^+CD25^-$ (DN1), $CD44^+CD25^+$ (DN2), $CD44^-CD25^+$ (DN3), and $CD44^-CD25^-$ (DN4).⁸ Mouse γ/δ T-cell development initiates in the thymus at the embryonic stage,

with some subsets of γ/δ T cells developing in an overlapping sequence. Unlike α/β T cells that are functionally differentiated to IL-17-producing effector cells only in the periphery, γ/δ T cells produce IL-17 even in the early fetal thymus.⁹ Transcription factor (TF) Sox4, Sox13, and HEB have been shown to be a master regulator of γ/δ T-cell differentiation in the thymus and to pivotally participate in the differentiation of IL-17-producing γ/δ T cells.¹⁰⁻¹² Although TFs and cytokines that regulate γ/δ T cells have been identified,¹³ the effects of lipid metabolism on T-cell differentiation have not been fully elucidated.

Fatty acids (FAs) are a critical source of energy for maintaining activation, proliferation, and function of lymphocytes.¹⁴ Although the immunological effects of polyunsaturated fatty acids (PUFAs) have been studied, the molecular mechanisms underlying these effects are still unknown. Fatty acid-binding proteins (FABPs) can bind to long-chain FAs including PUFAs and control signal transduction and transcription.¹⁵ FABP3 that preferably binds to ω -6 PUFA is widely expressed throughout the tissues and organs¹⁶⁻¹⁸ and recently shown to be expressed in helper T subsets.¹⁹ Regarding the function of FABPs in the immune cells, it is noted that tissue-resident memory T cells can survive for a long time by modifying their intracellular lipid environment in accordance with expressing FABP4 and FABP5 at the differentiation stages.²⁰ On the other hand, FABP4 and FABP5 have been reported to be involved in the regulation of asthma and skin inflammation,²¹⁻²³ suggesting that FABP molecules regulate allergic diseases through immune regulation linked to lipid metabolism. Therefore, it seems likely that FABP3 plays a critical role in the immunological function of lymphocytes and is involved in

the regulation of allergic diseases, but the roles of FABP3 in immune cells or regulation of allergic diseases are ill-defined.

In this study, we investigated the effects of FABP3-mediated changes in lipid metabolism of γ/δ T cells. We demonstrate a novel function of FABP3 as a negative regulator of $V\gamma 4^+$ γ/δ T cells development from thymic DN2 precursors.

2 | MATERIALS AND METHODS

2.1 | Cell culture

OP9/DII1 cells were kindly gifted by Dr Akihiko Yoshimura (Keio University, Japan). OP9 cells were cultured in MEM-alpha supplemented with 20% Fetal calf serum, 50 $\mu\text{mol/L}$ 2-mercaptoethanol, 2 mmol/L L-glutamine, and 1% penicillin/streptomycin. OP9/DII1 co-cultures were conducted in accordance with minor modifications of the previous protocol.²⁴ For DN cell development, thymic DN2 cells were placed on monolayers of OP9/DII1, supplemented with 5 ng/mL IL-7 and Flt3 ligand. After 4 days, the medium was replaced, and then, the cells were further incubated for 4 days more. For in vitro T-cell culture, naive ($\text{CD}44^{\text{lo}}\text{CD}62\text{L}^{\text{hi}}$) $\text{CD}4^+$ T cells were purified from spleens of wild-type or *Fabp3*^{-/-} mice with a naive $\text{CD}4^+$ T-cell isolation kit II (130-093-227; Miltenyi Biotec). Naive $\text{CD}4^+$ T cells were cultured with plate-bound anti-CD3 (1 $\mu\text{g/mL}$) and soluble anti-CD28 (1 $\mu\text{g/mL}$), with the addition of cytokines shown in Table S1.

2.2 | Mice

Fabp3^{-/-} mice on a C57BL/6 background have been described.²⁵ $\text{CD}45.2^+$ wild-type and *Fabp3*^{-/-} mice of the same genetic background were used in this study. All mice were bred and maintained under specific pathogen-free conditions at the Institute for Animal Experimentation, Tohoku University Graduate School of Medicine, and were used at the age of 5 days old or 8-12 weeks old. All procedures were approved by the Institutional Committee for the Use and Care of Laboratory Animals of Tohoku University.

2.3 | Induction of contact hypersensitivity

Two days after shaving the back hair with electrical clippers, the back skin was treated with 25 μL of 0.5% DNFB in a 4:1 mixture of acetone and olive oil or 150 μL of 1.0% FITC isomer I in a 1:1 mixture of acetone and dibutyl phthalate. Five days later, the respective mice were challenged with 40 μL of 0.2% DNFB or 0.5% FITC isomer I (each surface of the left ear) and 40 μL of the vehicle alone (each surface of the right ear). The ear thickness was measured before and after DNFB or FITC challenge using dial thickness gauges.

2.4 | RNA extraction and Real-time RT-PCR

Total RNA was extracted from whole skin tissues or sorted $V\gamma 4^+$ γ/δ T cells using RNeasy Micro Kit (QIAGEN) and reverse-transcribed with SuperScript III Reverse Transcriptase (Thermo Fisher Scientific). Real-time PCR was performed utilizing SYBR Premix Ex Tag (Takara Bio) on a 7500 real-time PCR system (Thermo Fisher Scientific). Relative gene expression was calculated by the ΔCt method and normalized to the amount of *Gapdh*. Primers were shown in Table S2.

2.5 | Flow cytometry

Cells were incubated with anti-CD16/CD32 (2.4G2) before being stained with the following antibodies to cell-surface and intracellular antigens. For intracellular staining, after cell activation with PMA/Ionomycin and staining of surface markers, cells were fixed and permeabilized with Cytofix/Cytoperm and Perm/Wash buffer (BD Biosciences) according to the manufacturer's instructions: CD3 (17A2), CD4 (GK1.5), CD8 (53-6.7), CD25 (PC61), CD44 (IM7), c-kit (2B8), γ/δ TCR (GL3), $V\gamma 4$ (UC3-10A6), $V\gamma 5$ (536), IL-17 (TC11-18H10.1), and IFN- γ (XMG1.2) (BioLegend). Data were acquired on a FACSCanto II (BD Biosciences) and were analyzed with FlowJo software (Tree Star).

2.6 | Retroviral transfection

Retroviral transduction was previously described²⁶ and was modified slightly. The cDNA that encodes FABP3 was cloned into pMY-IRES-EGFP. Recombinant retroviruses were prepared by transfecting the Plat-E packaging cells. Virus-containing supernatants were concentrated by centrifugation at 6000 $\times g$ at 4°C for 16 hours, and then, the concentrated virus particles were added into sorted DN2 cells from wild-type and *Fabp3*^{-/-} mice in the presence of 20 $\mu\text{g/mL}$ polybrene. Virus-containing media were replaced by fresh media, and the cells were used for experiments 24-48 hours later. In some experiments, FABP3-transduced DN2 cells were cultured over OP9/DII1 cells and were analyzed the capacity of differentiation into γ/δ T cells.

2.7 | Histochemistry and Immunofluorescence staining

For HE staining, ear tissues were fixed by ALTFIX and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin. For immunofluorescence staining, the thymus was fixed with ALTFIX and embedded in OCT compound. Sections were stained with antibodies as follows. The first and secondary antibodies were FABP3 (mouse polyclonal antibody) and goat anti-mouse IgG-Alexa Fluor 488 (Life Technologies), respectively. Nuclei were stained by DAPI (Life Technologies).

2.8 | Statistical analysis

To test differences among more than two groups, one-way analysis of variance (ANOVA) was used to establish statistical significance. In all other instances, an unpaired and two-tailed Student's *t* test was applied. *P* values of <.05 were considered statistical significance. **P* < .05, ***P* < .01, ****P* < .001.

3 | RESULTS

3.1 | FABP3-deficient mice display augmented contact hypersensitivity

To understand whether FABP3 regulates skin inflammatory responses, we first evaluated ear swelling after eliciting DNFB-induced CHS. *Fabp3*^{-/-} mice exhibited more severe ear swelling when compared with wild-type mice (Figure 1A). Histological analysis revealed hyperplasia of both epidermal and dermal skin in *Fabp3*^{-/-} mice (Figure 1B). Similarly, FITC-challenged *Fabp3*^{-/-} mice exhibited more severe ear swelling (Figure 1C). Moreover, FACS analysis showed that eosinophils and neutrophils, but not CD4⁺ T cells and CD8⁺ T cells, were more highly accumulated in *Fabp3*^{-/-} skin than wild-type (Figure 1D). CHS is regulated by adaptive immunity such as CD4⁺ helper T cells (Th) and CD8⁺ cytotoxic T cells (Tc).^{27,28} To examine the direct effect of FABP3-deficient pathogenic T cells, we performed CHS to congenic wild-type mice (CD45.1), which were transferred with sensitized wild-type or *Fabp3*^{-/-} α/β T cells (CD45.2). No difference in ear swelling between the two groups was observed (Figure 1E), suggesting adaptive immunity has no major role in the enhanced CHS phenotype observed in *Fabp3*^{-/-} mice. Additionally, mRNA expression of *Ifnγ* and *Il17a*, which play critical roles for skin inflammation,²⁹ was elevated in the inflamed skin of *Fabp3*^{-/-} mice (Figure 1F). However, there was no significant difference in the expression of *Il4*, *Il13*, *Cxcl1*, *Cxcl2*, *Ccl2*, and *Ccl20* mRNA between *Fabp3*^{-/-} mice and wild-type mice (Figure 1F). These results suggested that FABP3 negatively regulates CHS probably by modulating inflammatory responses.

3.2 | FABP3-deficient mice exhibited normal T-cell development

Although Figure 1 results showed there was no difference between the two groups in the number of infiltrating T cells into the inflamed ear tissues, it is unclear whether there is a functional difference between wild-type and *Fabp3*^{-/-} T cells. As a result, *Fabp3*^{-/-} mice display normal T-cell populations in spleen, LNs, and thymus (Figure S1A-C). Although Th1 and Th17 highly expressed FABP3 when compared with naive CD4⁺ T cells,¹⁹ *Fabp3*^{-/-} naive CD4⁺ T cells could normally differentiate into T-bet⁺ and IFNγ⁺ (Th1), GATA3⁺, IL-5⁺ and IL-13⁺ (Th2), RORγt⁺ and IL-17A⁺ (Th17), and Foxp3⁺ (Treg) T cells when

compared with wild-type naive CD4⁺ T cells (Figure S1D-G). These results suggest that FABP3 has no critical effect on the differentiation and function of α/β T cells.

3.3 | Increase in γ/δ T cells in inflamed ear tissues of FABP3-deficient mice

In the skin, the epidermal layer exclusively contains Vγ5⁺ γ/δ T cells, while the dermal layer enriches Vγ4⁺ and other V chain positive γ/δ T cells.^{30,31} IL-17-producing γ/δ T cells in dermis mainly comprise of Vγ4⁺ γ/δ T cells, which are attributed to CHS regulation.^{5,32} To clarify whether dermal Vγ4⁺ γ/δ T cells are responsible for the augmented skin inflammation in *Fabp3*^{-/-} mice, we analyzed inflamed ear tissues by flow cytometry. We found that there were significantly higher numbers of Vγ5⁺ and Vγ4⁺ γ/δ T cells accumulated in the skin of *Fabp3*^{-/-} mice when compared with that of wild-type mice after DNFB-challenge (Figure 2A, B). Furthermore, we found that *Fabp3*^{-/-} mice exhibited a more infiltration of IL-17-producing Vγ4⁺ γ/δ T cells in the inflamed ear tissues (Figure 2C). In accordance with intracellular cytokine staining, *Fabp3*^{-/-} Vγ4⁺ γ/δ T cells produced a high amount of IL-17A compared with wild-type cells (Figure S2). On the contrary, there was no difference in the number of IFN-γ-producing Vγ5⁺ γ/δ T cells between the two groups (Figure S3).

While CCR6 is involved in the positioning of IL-17-producing γ/δ T cell in the skin at steady state, CCR2 controls the migration of these cells into inflamed skin tissues.^{33,34} In this process, activated γ/δ T cells downregulate CCR6 while upregulating CCR2.³³ To evaluate the effect of FABP3 deficiency on these chemokine receptors, we analyzed *Ccr6* and *Ccr2* mRNA expression in Vγ4⁺ γ/δ T cells by quantitative PCR. Interestingly, sorted dermal *Fabp3*^{-/-} Vγ4⁺ γ/δ T cells expressed higher levels of *Ccr6* and *Ccr2* when compared with wild-type cells (Figure 2D). In addition, sorted dermal *Fabp3*^{-/-} Vγ4⁺ γ/δ T cells also exhibited higher levels of *Heb*, *Sox4*, *Sox13*, *Rorc*, and *Maf* (Figure 2E). Previous studies have shown that *Heb*, *Sox4*, *Sox13*, *Rorc*, and *Maf* interact with each other to regulate mutually these genes expression in γ/δ T cells,^{12,31,35} and that *Rorc* expression regulates IL-17A production and CCR6 expression, while *Maf* potentially controls CCR2 expression.^{36,37} Thus, our results, in line with these findings, suggest FABP3 regulates γ/δ T-cell activity through probably regulating the expression of their essential genes.

3.4 | FABP3 deficiency affects γ/δ T-cell distribution in the periphery

Since we have shown that FABP3 is involved in the expression of molecules that control the dynamics of Vγ4⁺ γ/δ T cells, we next evaluated the distribution of this cell type in various FABP3-deficient organs. Although the percentage of total γ/δ T cells was no difference between two groups (Figure 3A-E), there was a higher

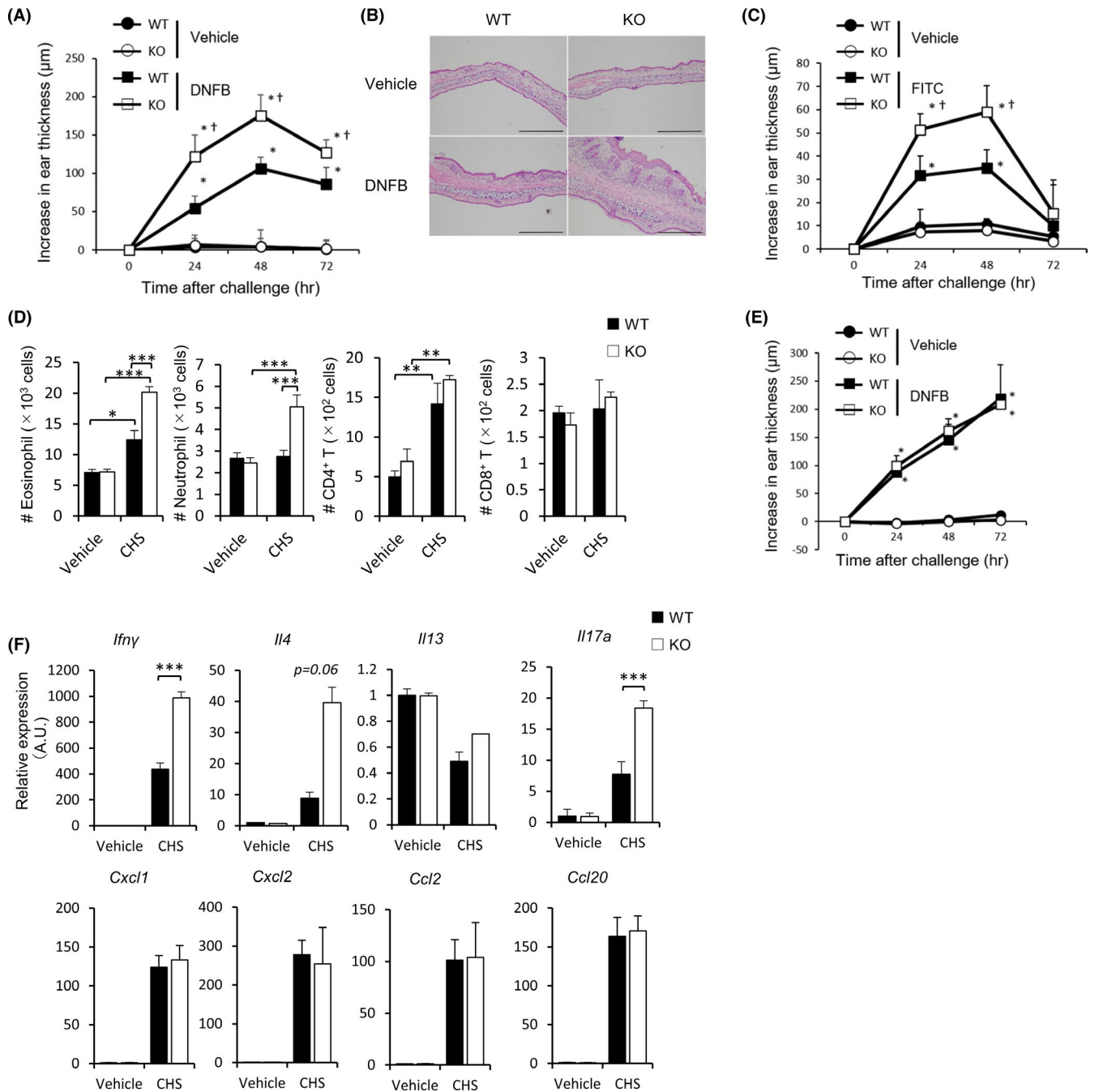


FIGURE 1 FABP3 limits contact hypersensitivity. (A) Wild-type (WT) and *Fabp3*^{-/-} (KO) mice were sensitized and challenged with DNFB. The ear skin thickness of the mice was measured at the indicated time points after challenge with DNFB or vehicle alone. * $P < .05$ vs. corresponding values for vehicle-treated mice, and † $P < .05$ vs. hapten-challenged wild-type mice. (B) Representative histological views (H&E) of ear skin from WT and KO mice at 24 h after challenge with DNFB or vehicle alone. Scale bar = 50 μm . (n = 8) (C) WT and KO mice were sensitized and challenged with FITC. The ear skin thickness of the mice was measured at the indicated time points after challenge with FITC or vehicle alone. * $P < .05$ vs. corresponding values for vehicle-treated mice, and † $P < .05$ vs. hapten-challenged wild-type mice. (D) The absolute number of eosinophils, neutrophils, CD4^+ T cells, and CD8^+ T cells in indicated skin samples at 48 h after challenge. (E) WT and KO mice (CD45.2) were sensitized with DNFB. Five days after sensitization, purified α/β CD3^+ T cells (5.0×10^6 cells) from DNFB-sensitized mice were transferred to naive congenic WT mice (CD45.1), and these mice were challenged with DNFB or vehicle alone 24 h after cell transfer. The ear skin thickness of the mice was measured at the indicated time points after challenge with DNFB or vehicle alone. * $P < .05$ vs. corresponding values for vehicle-treated mice. (F) *Ifn γ* , *Il17a*, *Il4*, *Il13*, *Cxcl1*, *Cxcl2*, *Ccl2*, and *Ccl20* mRNA expression in whole ear skin. Results are normalized to those of *Gapdh* and are presented relative to those of vehicle-challenged WT mice. * $P < .05$, *** $P < .001$ (Student's *t* test). Data are from one experiment representative of three independent experiments with similar results (A, C, D) (mean \pm SEM of total 12 mice per genotype (A, C, E); mean \pm SEM of three replicates (D, F))

percentage of $V\gamma 4^+ \gamma/\delta$ T cells in the skin but significant lower percentages of the cell type in the thymus, spleen, and inguinal LNs (iLNs) in FABP3-deficient mice when compared with wild-type mice (Figure 3A, F). Nevertheless, $V\gamma 4^+ \gamma/\delta$ T cells in peripheral blood were comparable between WT and *Fabp3*^{-/-} mice (Figure 3F). γ/δ T cells arise from common multipotent double-negative (DN) precursors in the thymus, which can be further dissected into four subsets based on CD44 and CD25 expression.^{38,39} Contingent on the previous findings in adult mice, we hypothesized that FABP3 deficiency might affect the generation of $V\gamma 4^+ \gamma/\delta$ T cells at the fetal stage. By using juvenile mice at five days old, we identified that juvenile *Fabp3*^{-/-} mice displayed more $V\gamma 4^+ \gamma/\delta$ T cells than wild-type counterparts in thymus and skin tissues (Figure 4A, B), while no difference in the number of total thymocytes (Figure 4C). Moreover, sorted dermal $V\gamma 4^+ \gamma/\delta$ T cells in juvenile *Fabp3*^{-/-} mice expressed high levels of *Ccr6*, *Ccr2*, *Heb*, *Sox4*, *Sox13*, *Rorc*, and *Maf*, which is also observed in adult mice (Figure 4D, E). These results suggest that *Fabp3*^{-/-} mice had increased generation of $V\gamma 4^+ \gamma/\delta$ T cells highly expressed these genes in the thymus during at least neonatal stage, leading to an increased proportion of $V\gamma 4^+ \gamma/\delta$ T cells in the skin.

3.5 | FABP3 is expressed by thymic DN2 precursors and regulates its differentiation into $V\gamma 4^+ \gamma/\delta$ T cell

Given that FABP3 deficiency has an impact on γ/δ T-cell distribution, we sought to assess the expression of FABP3 in the thymus. IL-17-producing γ/δ T cells (mainly $V\gamma 4^+$) have shown to be derived from thymic DN2 cells.⁴⁰ Fluorescent immunostaining demonstrated that FABP3 localizes in the thymic cortex (Figure 5A). In line with that, thymic DN2 cells abundantly express *Fabp3* when compared with thymic cells (Figure 5B). $V\gamma 4^+ \gamma/\delta$ T cells are known to be produced from DN2 precursors in the thymus during the fetal/neonatal stage and mainly distribute to the skin dermal layer.³¹ Juvenile *Fabp5*^{-/-} mice showed a higher frequency of DN2 cells in the thymus (Figure 5C), suggesting that FABP3 controls differentiation in DN stages. To further evaluate the intrinsic developmental potential of DN precursors under the same conditions, we next conducted the OP9-DL1 co-culture system.⁴¹ Double-negative 2 cells were sorted and placed in the OP9-DL1 co-culture with recombinant IL-7 and Flt3 ligand. Interestingly, there was a significantly higher percentage of $V\gamma 4^+ \gamma/\delta$ T cells differentiated from FABP3-deficient DN2 when compared with wild-type DN2 cells (Figure 5D). Moreover, to obtain the direct evidence of FABP3 in intrinsically controlling the differentiation of DN2 cells into $V\gamma 4^+ \gamma/\delta$, we infected *Fabp3*^{-/-} DN2 cells with the FABP3-encoding retrovirus vector. We found that FABP3-transgene thymic *Fabp3*^{-/-} DN2 cells illustrated a normal differentiation into $V\gamma 4^+ \gamma/\delta$ T cells (Figure 5E). Finally, we observed that *Fabp3*^{-/-} DN2 cells displayed a higher expression of *Heb*, *Sox4*, and *Sox13*, when compared with wild-type DN2, and transgene of FABP3 to *Fabp3*^{-/-} DN2 cells normalized its abnormal expression

(Figure 5F). These results suggest FABP3 in thymic DN2 cells intrinsically plays an important role in appropriate commitment of γ/δ T cells.

4 | DISCUSSION

The skin resident immune cells work together closely to maintain homeostasis of skin tissues.^{42,43} Obesity has been suspected as a major risk factor of allergic diseases, including asthma, atopic dermatitis, CHS, and psoriasis.⁴⁴⁻⁴⁷ Previous reports provide many pieces of evidence that obesity is tightly involved in skin inflammation through functional changes in immune cells.^{23,48} As described above, γ/δ T cells also are reported to regulate the skin inflammation.^{7,49} In line with previous reports, *Fabp3*^{-/-} mice exhibited a severe phenotype of CHS accompanied by hyper-activation of IL-17-producing $V\gamma 4^+ \gamma/\delta$ T cells. Surprisingly, our results revealed that thymic DN2 cells were found to express FABP3 abundantly, and FABP3 regulates the development of $V\gamma 4^+ \gamma/\delta$ T cells from these cells in the thymus. Together these findings clarify a previously unknown function of FABP3 in pathogenic $V\gamma 4^+ \gamma/\delta$ T-cell development.

Although γ/δ T cells are a minor population compared to α/β T cells, these cells are involved in various inflammations.⁵⁰ Unlike α/β T cells, however, few reports are showing the relationship between lipid metabolism and γ/δ T cells. PUFA-enriched diet-fed mice exhibited a large number of splenic γ/δ T cells and high-fat diet-fed mice exhibited exacerbated dermatitis accompanied by accumulation of IL-17-producing $V\gamma 4^+ \gamma/\delta$ T cells in the skin,⁷ suggesting that changes in the lipid environment certainly have some effect on γ/δ T cells. Besides, γ/δ T cells were reported to contain a higher cholesterol level than α/β T cells, and the increase in intracellular cholesterol of γ/δ T cells affects the activation of these cells by enhancing the basal T-cell receptor intensity.⁵¹ In *Fabp3*^{-/-} γ/δ T cells, it is conceivable that down-regulation of intracellular cholesterol level due to a disruption of the ω -6/ ω -3 ratio by the decrease in intracellular ω -6 PUFA, resulting in enhancing the activation of these cells. Meanwhile, γ/δ T cells are also known to be epigenetically regulated, as well as α/β T cells.⁵² Thymus development of IL-17-producing γ/δ T cells is restricted to the embryonic and perinatal stages during embryonic development,⁹ and it is demonstrated that these epigenetic regulations in γ/δ T cells have already occurred in the thymus.⁵³ Additionally, Malhotra et al have reported the histone modification profile of *Rorc* locus in immature $V\gamma 4^+$ thymocytes was active condition.¹² This knowledge suggests that changes in the thymic lipid microenvironment caused by FABP3 deficiency affect the epigenetic regulation in early γ/δ T-cell differentiation. Interestingly, we found that despite there was no difference in percentages of total skin γ/δ T cells between *Fabp3*^{-/-} mice and wild-type mice at steady state, a higher percentage of $V\gamma 4^+ \gamma/\delta$ T cells was present in *Fabp3*^{-/-} skin accompanied with high expression of chemokine receptors and TFs. Furthermore, since juvenile *Fabp3*^{-/-} skin also demonstrated a higher percentage of $V\gamma 4^+ \gamma/\delta$

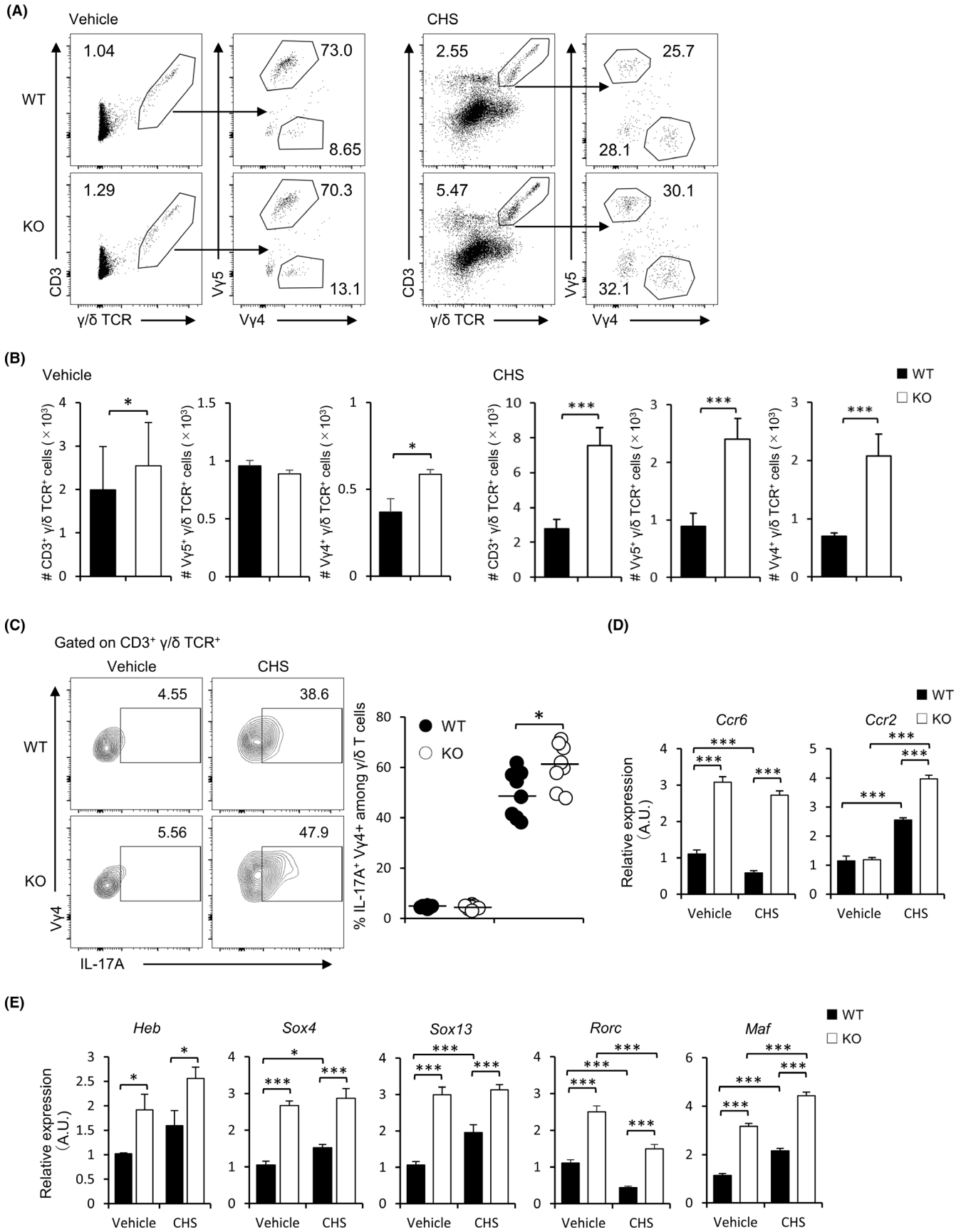


FIGURE 2 Exacerbation of CHS is associated with skin $V\gamma 4 + \gamma/\delta$ T cells increment in *Fabp3*^{-/-} mice. (A, B) Frequency (left) and number (right) of $CD3^+ \gamma/\delta TCR^+$, $V\gamma 5^+$ and $V\gamma 4^+ \gamma/\delta$ T cells in the inflamed (DNFB) ears (A) and normal (vehicle) ears (B) of WT and *Fabp3*^{-/-} mice at 24 h after challenge. (C) Frequency (left) and number (right) of $IL-17A^+ V\gamma 4^+ \gamma/\delta$ T cells in the inflamed ears of WT and KO mice. (D, E) *Ccr6* and *Ccr2* (D) and *Heb*, *Sox4*, *Sox13*, *Rorc*, and *Maf* (E) mRNA expression of sorted dermal $V\gamma 4^+ \gamma/\delta$ T cells in the inflamed and normal skin. Results are normalized to those of *Gapdh* and are presented relative to those of sorted dermal $V\gamma 4^+ \gamma/\delta$ T cells in vehicle-challenged WT mice. * $P < .05$ and *** $P < .001$ (Student's *t* test). Data are from one experiment representative of three independent experiments with similar results (A-E) (mean \pm SEM of three replicates (A-E))

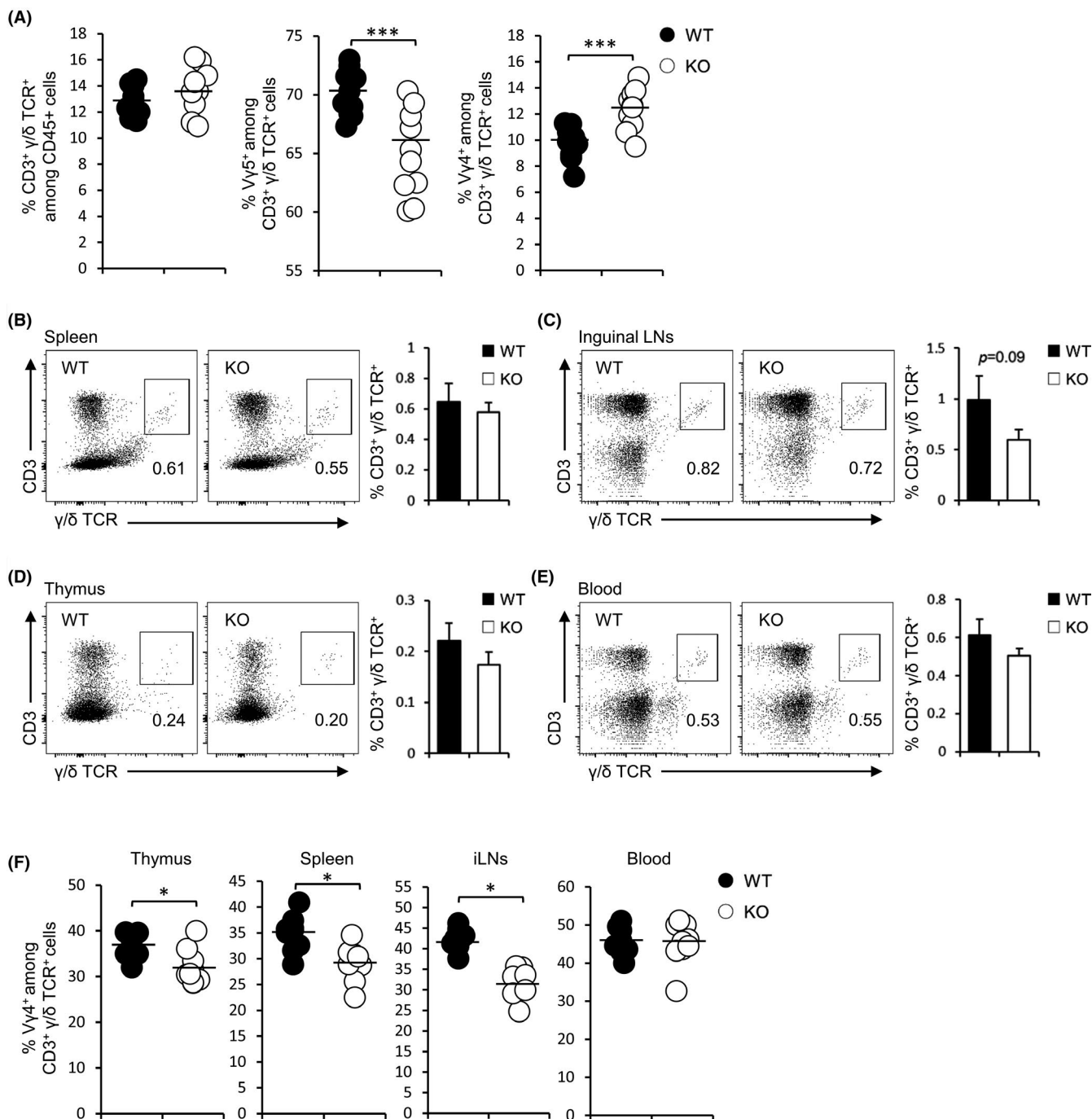


FIGURE 3 FABP3-deficient adult mice displayed a high percentage of $V\gamma 4 + \gamma/\delta$ T cells in the skin, but not thymus, spleen, inguinal lymph nodes, and peripheral blood. (A) Percentages of $CD3^+ \gamma/\delta TCR^+$ cells, $V\gamma 5^+$, and $V\gamma 4^+ \gamma/\delta$ T cells in the ear skin of adult WT and KO mice. (B-E) Total $CD3^+ \gamma/\delta$ T cell profiles in the spleen (B), iLNs (C), thymus (D), and peripheral blood (E) from WT and KO mice. Numbers adjacent to outlined areas (left) indicate percentages of $CD3^+$ and $\gamma/\delta TCR^+$ T cells. (F) Percentages of $V\gamma 4^+ \gamma/\delta$ T cells in various tissues of WT and KO mice. * $P < .05$, *** $P < .001$ (Student's *t* test). Data are one experiment representative of at least three independent experiments with similar results (mean \pm SEM of three replicates (A-F))

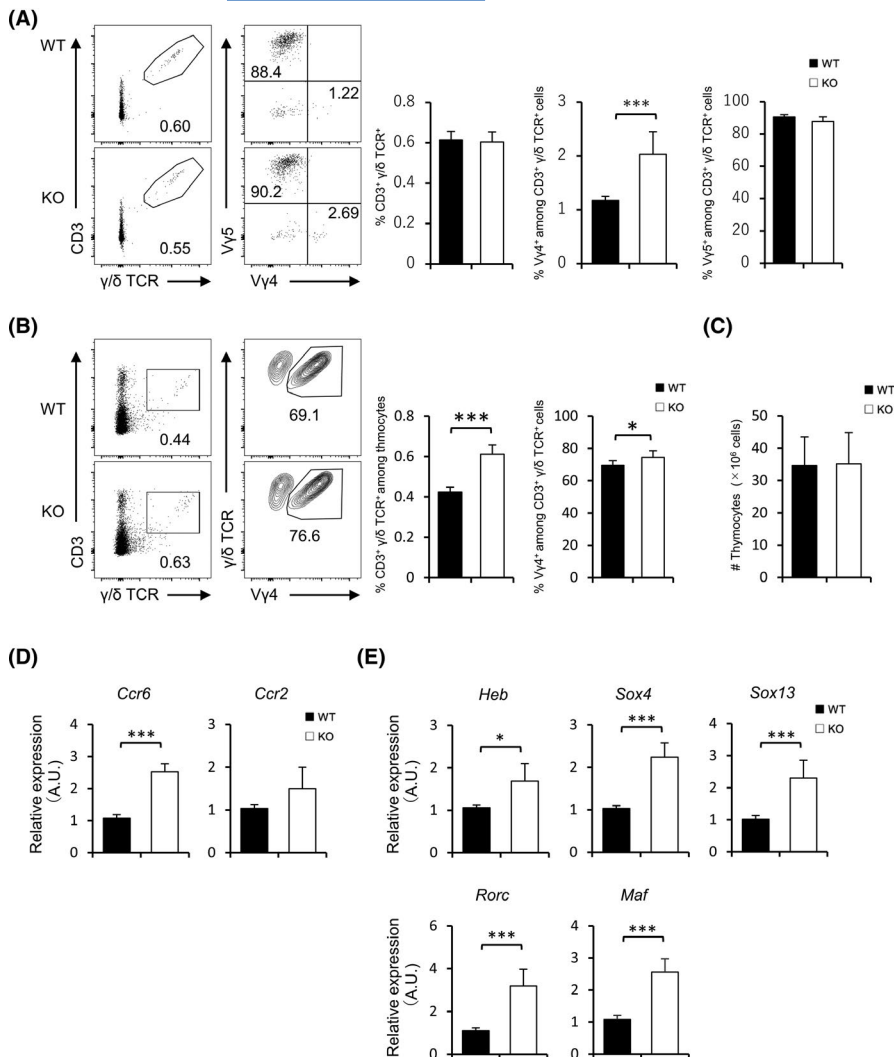


FIGURE 4 Juvenile FABP3-deficient mice showed more abundant Vγ4 + γ/δ T cells. (A, B) Frequency (left) and number (right) of CD3⁺ γ/δ TCR⁺, Vγ5⁺, and Vγ4⁺ γ/δ T cells in the skin (A) and thymus (B) of juvenile WT and *Fabp3*^{-/-} mice. (C) The number of thymocytes in juvenile WT and *Fabp3*^{-/-} mice. (D, E) *Ccr6* and *Ccr2* (D) and *Heb*, *Sox4*, *Sox13*, *Rorc*, and *Maf* (E) mRNA expression of sorted dermal Vγ4⁺ γ/δ T cells in juvenile mice. Results are normalized to those of *Gapdh* and are presented relative to those of WT Vγ4⁺ γ/δ T cells. **P* < .05, ****P* < .001 (Student's *t* test). Data are from one experiment representative of three independent experiments with similar results (A-E) (mean ± SEM of three replicates (A-E))

T cells, we speculated that FABP3 might involve in the early γ/δ T-cell formation in thymus.

The thymus is an important organ for T-cell development.⁵⁴ A body of reports revealed a requirement of IL-7 in the proper IL-17-producing γ/δ T-cell development.^{55,56} Notch ligand expressed by thymic epithelial cells has also been suggested to be involved in IL-17-producing γ/δ T-cell differentiation.⁵⁷ Additionally, IL-17-producing γ/δ T cells are considered to be generated from thymic T-cell precursors which receive weak TCR signals during their development.⁵⁸ Although the mechanisms of γ/δ T-cell commitment regulated by cytokines, stimulatory molecules, and TFs have been elucidated, how the lipid environment in thymus affects T-cell development remains poorly unveiled. Many studies demonstrated that obesity promotes thymus senescence accompanied by affecting thymic epithelial cells and T-cell precursors.^{59,60} Moreover, aging alters the composition of γ/δ T cells in peripheral blood and LNs, resulting in an increase in IL-17-producing γ/δ T cells including Vγ4⁺ γ/δ T cells.^{61,62} Our results revealed that *Fabp3*^{-/-} mice displayed a large number of Vγ4⁺ γ/δ T cells in the juvenile thymus. It is also noteworthy that the FA composition of the thymus has

been shown to be rich in ω-6 PUFA,⁶³ to which FABP3 prefers to bind. We previously showed that FABP3 is involved in epigenetic regulation in B cells.⁶⁴ Furthermore, PUFAs have been reported to be responsible for epigenetic modifications.^{65,66} Our results from the OP9-DL1 co-culture system indicate that these events are caused by the intrinsic effects of FABP3 in DN2 cells. Considering above, changes in the thymic lipid microenvironment during fetal γ/δ T-cell development in *Fabp3*^{-/-} mice affected the balance in γ/δ T-cell commitment through modulating TF expression by epigenetic modifications in thymic DN2 cells due to some triggered induced by an abnormality in the suitable PUFA balance. Furthermore, our current results suggest that changes in the lipid microenvironment during the fetal period may regulate the commitment of pathogenic γ/δ T-cell development in the thymus and affect the incidence of allergic dermatitis in adults. We hypothesize that abnormal maternal fatty acid intake causes disruption of lipid homeostasis during the fetal or neonatal period and promotes the production of pathogenic Vγ4⁺ γ/δ T cells in the skin, which in turn influences the development of allergic skin inflammation in adulthood. Therefore, we also infer that proper regulation of

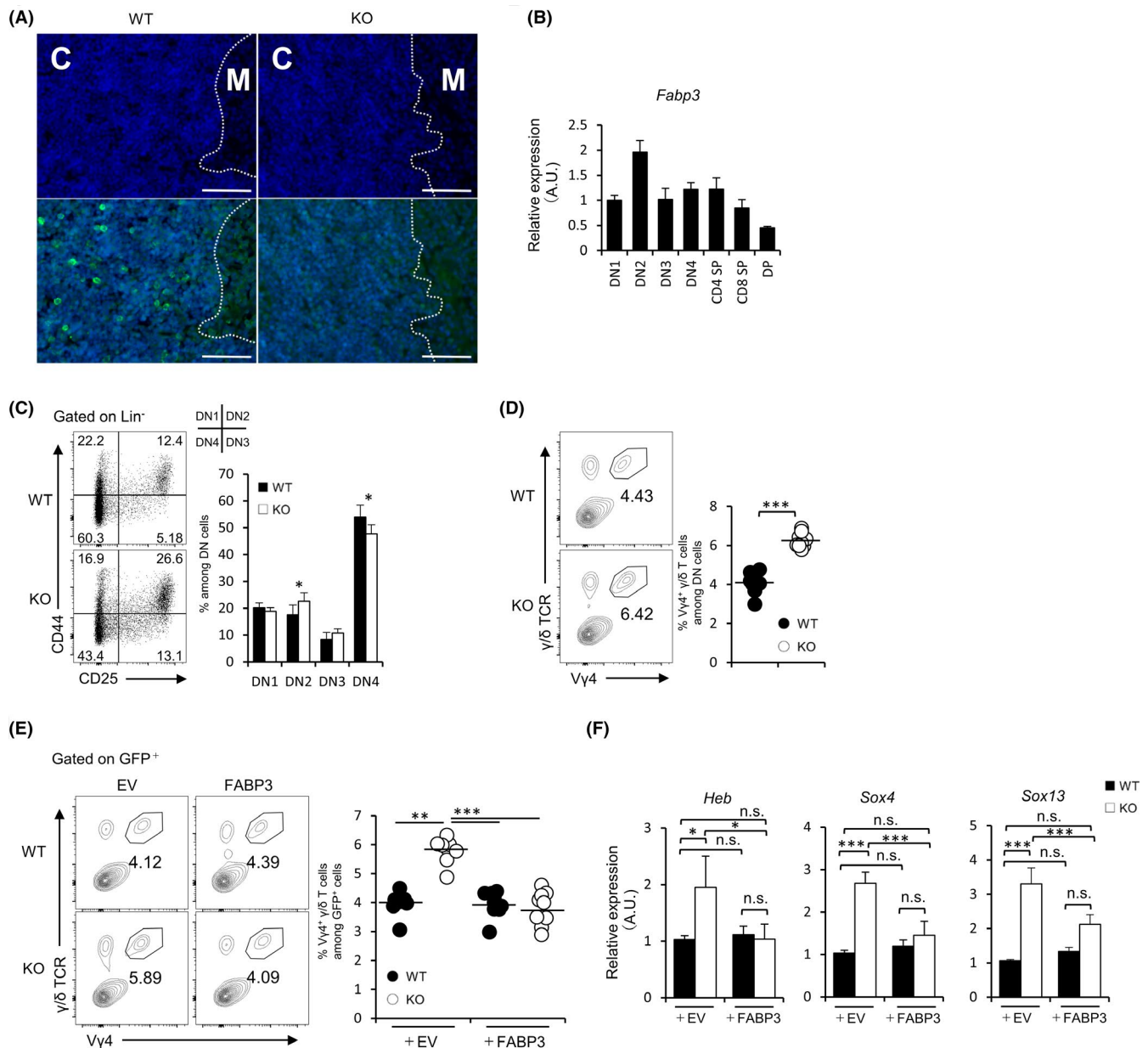


FIGURE 5 FABP3 regulates differentiation thymic DN2 cells into V γ 4⁺ γ / δ T cells. (A) Immunofluorescence staining of FABP3 (green) and DAPI (blue) in the thymus. C: cortex, M: medulla, Scale bar: 50 μ m. (B) *Fabp3* mRNA expression of thymic double-negative cells (DN), CD4 single-positive thymocyte (CD4 SP), CD8 single-positive thymocyte (CD8 SP), and double-positive cell (DP) (C) Frequency (left) and number (right) of thymic DN1 (CD44⁺CD25⁻), DN2 (CD44⁺CD25⁺), DN3 (CD44⁻CD25⁺), and DN4 (CD44⁻CD25⁻) cells in juvenile WT and KO mice. (D) Sorted DN2 cells from juvenile WT and KO mice were co-cultured with OP9/DII1 cells in the presence of IL-7 (5 ng/mL) and Flt3 ligand (5 ng/mL). Representative dot plots show the frequency of V γ 4⁺ γ / δ T cells while graphs indicate the number of V γ 4⁺ γ / δ T cells. (E) WT and KO DN2 cells were retrovirally transduced with FABP3, and GFP⁺ DN2 cells were co-cultured on OP9/DII1 cells with IL-7 (5 ng/mL) and Flt3 ligand (5 ng/mL). Representative dot plots show the frequency of GFP⁺ V γ 4⁺ γ / δ T cells while graphs indicate the number of GFP⁺ V γ 4⁺ γ / δ T cells. (F) *Heb*, *Sox4*, and *Sox13* mRNA expression of thymic DN2 cells in juvenile mice. Results are normalized to those of *Gapdh* and are presented relative to those of WT transducing EV. EV: empty vector, * P < .05, *** P < .001 (Student's t test). n.s. not significant. Data are from one experiment representative of three independent experiments with similar results (A-F) (mean \pm SEM of three replicates (B-F))

fatty acid intake (or composition) in the motherhood may be able to control some of the onset and pathological exacerbations of skin allergic inflammation in the offspring.

Some studies reported that lipid metabolism in α/β T cells plays important role in generating memory T cells^{67,68} and that the metabolic pattern dramatically changes when transitioning from naïve T

cells to effector T cells and from effector T cells to memory T cells.⁶⁹ Recently, FABP3 was shown to be abundantly expressed by Th17 compared with naïve CD4⁺ T cells.¹⁹ However, our current results demonstrated that FABP3-deficient naïve CD4⁺ T cells exhibited a comparable capacity of Th17 differentiation when compared with wild-type counterparts. Since Th17 drastically changes its activity

due to arachidonic acid and/or arachidonic acid metabolites,^{70,71} it is possible that FABP3, which preferably binds to arachidonic acid,¹⁶ is involved in functional expression of Th17 cells, including memory T cells formation, but not Th17 differentiation. Interestingly, since it has been reported that FABP4/5, which is highly homologous to FABP3, regulates longevity of tissue-resident memory T cells,²⁰ FABP3 maybe participate in memory T-cell regulation by linking with ω -6 PUFA. Further consideration will be needed to yield any findings about this point.

In conclusion, we have revealed a critical role of FABP3 in regulating γ/δ T-cell development and activity via modulating the expression of TFs in the thymus. This study demonstrates a novel mechanism in skin inflammation that illustrates a linkage between lipid metabolism and γ/δ T-cell-mediated responses and thus provides a new therapeutic approach to treat several immune diseases.

ACKNOWLEDGMENTS

We thank the Biomedical Research Core and the Institute for Animal Experimentation (Tohoku University Graduate School of Medicine) for technical support. This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant (No. 19K24307 and 20K16392 to SK, 19H04026 to Y. O.) and by AMED under Grant Number JP17dm0107071 (to KF and YO).

CONFLICT OF INTEREST

The authors have no financial conflicts of interest.

AUTHOR CONTRIBUTIONS

SK and YO conceived and directed this study, designed and performed experiments, analyzed the data, and contributed to the writing of the manuscript. HP, YK, HM, AA, and YT performed experiments and analyzed the data. TM, YA, and NI provided critical materials, discussion, participated in the interpretation of the data, and contributed to the writing of the manuscript.

ORCID

Shuhei Kobayashi  <https://orcid.org/0000-0002-1019-8669>

Hai The Phung  <https://orcid.org/0000-0002-0439-2588>

Yoshiteru Kagawa  <https://orcid.org/0000-0003-2963-7905>

Hirofumi Miyazaki  <https://orcid.org/0000-0003-2695-7479>

Takashi Maruyama  <https://orcid.org/0000-0003-0820-3219>

Naoto Ishii  <https://orcid.org/0000-0002-0549-8245>

REFERENCES

1. Ho AW, Kupper TS. T cells and the skin: from protective immunity to inflammatory skin disorders. *Nat Rev Immunol*. 2019;19(8):490-502.
2. Sun LD, Qiao S, Wang Y, et al. V γ 4+ T Cells: A Novel IL-17-Producing $\gamma\delta$ T Subsets during the Early Phase of Chlamydial Airway Infection in Mice. *Mediators Inflamm*. 2018;2018:6265746.
3. Chien YH, Meyer C, Bonneville M. $\gamma\delta$ T cells: first line of defense and beyond. *Annu Rev Immunol*. 2014;32:121-155.
4. Li Y, Wu J, Luo G, He W. Functions of V γ 4 T cells and dendritic epidermal T cells on skin wound healing. *Front Immunol*. 2018;9:1099.
5. Cai Y, Shen X, Ding C, et al. Pivotal role of dermal IL-17-producing $\gamma\delta$ T cells in skin inflammation. *Immunity*. 2011;35(4):596-610.
6. Mehta P, Nuotio-Antar AM, Smith CW. $\gamma\delta$ T cells promote inflammation and insulin resistance during high fat diet-induced obesity in mice. *J Leukoc Biol*. 2015;97(1):121-134.
7. Nakamizo S, Honda T, Adachi A, et al. High fat diet exacerbates murine psoriatic dermatitis by increasing the number of IL-17-producing $\gamma\delta$ T cells. *Sci Rep*. 2017;7(1):14076.
8. Germain RN. T-cell development and the CD4-CD8 lineage decision. *Nat Rev Immunol*. 2002;2(5):309-322.
9. Haas JD, Ravens S, Düber S, et al. Development of interleukin-17-producing $\gamma\delta$ T cells is restricted to a functional embryonic wave. *Immunity*. 2012;37(1):48-59.
10. Melichar HJ, Narayan K, Der SD, et al. Regulation of gammadelta versus alphabeta T lymphocyte differentiation by the transcription factor SOX13. *Science*. 2007;315(5809):230-233.
11. Gray EE, Ramirez-Valle F, Xu Y, et al. Deficiency in IL-17-committed V γ 4(+) $\gamma\delta$ T cells in a spontaneous Sox13-mutant CD45.1(+) congenic mouse substrain provides protection from dermatitis. *Nat Immunol*. 2013;14(6):584-592.
12. Malhotra N, Narayan K, Cho OH, et al. A network of high-mobility group box transcription factors programs innate interleukin-17 production. *Immunity*. 2013;38(4):681-693.
13. Baccala R, Witherden D, Gonzalez-Quintal R, et al. Gamma delta T cell homeostasis is controlled by IL-7 and IL-15 together with subset-specific factors. *J Immunol*. 2005;174(8):4606-4612.
14. Nicolaou A, Mauro C, Urquhart P, Marelli-Berg F. Polyunsaturated Fatty Acid-derived lipid mediators and T cell function. *Front Immunol*. 2014;5:75.
15. Hanhoff T, Lücke C, Spener F. Insights into binding of fatty acids by fatty acid binding proteins. *Mol Cell Biochem*. 2002;239(1-2):45-54.
16. Islam A, Kagawa Y, Sharifi K, et al. Fatty Acid Binding Protein 3 Is Involved in n-3 and n-6 PUFA transport in mouse trophoblasts. *J Nutr*. 2014;144(10):1509-1516.
17. Yamamoto Y, Kida H, Kagawa Y, et al. FABP3 in the anterior cingulate cortex modulates the methylation status of the glutamic acid decarboxylase. *J Neurosci*. 2018;38(49):10411-10423.
18. Zhuang L, Li C, Chen Q, et al. Fatty acid-binding protein 3 contributes to ischemic heart injury by regulating cardiac myocyte apoptosis and MAPK pathways. *Am J Physiol Heart Circ Physiol*. 2019;316(5):H971-H984.
19. Field CS, Baixauli F, Kyle RL, et al. Mitochondrial Integrity Regulated by Lipid Metabolism Is a Cell-Intrinsic Checkpoint for Treg Suppressive Function. *Cell Metab*. 2019;31(2):422-437.
20. Pan Y, Tian T, Park CO, et al. Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. *Nature*. 2017;543(7644):252-256.
21. Shum BO, Mackay CR, Gorgun CZ, et al. The adipocyte fatty acid-binding protein aP2 is required in allergic airway inflammation. *J Clin Invest*. 2006;116(8):2183-2192.
22. Suojalehto H, Kinaret P, Kilpeläinen M, et al. Level of Fatty Acid Binding Protein 5 (FABP5) Is Increased in Sputum of Allergic Asthmatics and Links to Airway Remodeling and Inflammation. *PLoS One*. 2015;10(5):e0127003.
23. Zhang Y, Li Q, Rao E, et al. Epidermal Fatty Acid binding protein promotes skin inflammation induced by high-fat diet. *Immunity*. 2015;42(5):953-964.
24. Wang H, Pierce LJ, Spangrude GJ. Distinct roles of IL-7 and stem cell factor in the OP9-DL1 T-cell differentiation culture system. *Exp Hematol*. 2006;34(12):1730-1740.
25. Binas B, Danneberg H, McWhir J, Mullins L, Clark AJ. Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. *FASEB J*. 1999;13(8):805-812.
26. Simmons A, Alberola-Ila J. Retroviral Transduction of T Cells and T Cell Precursors. *Methods Mol Biol*. 2016;1323:99-108.

27. Saint-Mezard P, Berard F, Dubois B, Kaiserlian D, Nicolas JF. The role of CD4+ and CD8+ T cells in contact hypersensitivity and allergic contact dermatitis. *Eur J Dermatol*. 2004;14(3):131-138.
28. Akiba H, Kehren J, Ducluzeau MT, et al. Skin inflammation during contact hypersensitivity is mediated by early recruitment of CD8+ T cytotoxic 1 cells inducing keratinocyte apoptosis. *J Immunol*. 2002;168(6):3079-3087.
29. He D, Wu L, Kim HK, Li H, Elmets CA, Xu H. IL-17 and IFN-gamma mediate the elicitation of contact hypersensitivity responses by different mechanisms and both are required for optimal responses. *J Immunol*. 2009;183(2):1463-1470.
30. O'Brien RL, Born WK. Dermal $\gamma\delta$ T cells—What have we learned? *Cell Immunol*. 2015;296(1):62-69.
31. Jouan Y, Patin EC, Hassane M, Si-Tahar M, Baranek T, Paget C. Thymic Program Directing the Functional Development of $\gamma\delta$ T17 Cells. *Front Immunol*. 2018;9:981.
32. Jiang X, Park CO, Geddes Sweeney J, Yoo MJ, Gaide O, Kupper TS. Dermal $\gamma\delta$ T Cells Do Not Freely Re-Circulate Out of Skin and Produce IL-17 to Promote Neutrophil Infiltration during Primary Contact Hypersensitivity. *PLoS One*. 2017;12(1):e0169397.
33. McKenzie DR, Kara EE, Bastow CR, et al. IL-17-producing $\gamma\delta$ T cells switch migratory patterns between resting and activated states. *Nat Commun*. 2017;8:15632.
34. Ramírez-Valle F, Gray EE, Cyster JG. Inflammation induces dermal V γ 4+ $\gamma\delta$ T17 memory-like cells that travel to distant skin and accelerate secondary IL-17-driven responses. *Proc Natl Acad Sci U S A*. 2015;112(26):8046-8051.
35. In TSH, Trotman-Grant A, Fahl S, et al. HEB is required for the specification of fetal IL-17-producing $\gamma\delta$ T cells. *Nat Commun*. 2017;8(1):2004.
36. Skepner J, Ramesh R, Trocha M, et al. Pharmacologic inhibition of ROR γ t regulates Th17 signature gene expression and suppresses cutaneous inflammation in vivo. *J Immunol*. 2014;192(6):2564-2575.
37. Wheaton JD, Yeh CH, Ciofani M. Cutting Edge: c-Maf Is Required for Regulatory T Cells To Adopt ROR γ t+ and Follicular Phenotypes. *J Immunol*. 2017;199(12):3931-3936.
38. Allman D, Sambandam A, Kim S, et al. Thymopoiesis independent of common lymphoid progenitors. *Nat Immunol*. 2003;4(2):168-174.
39. Porritt HE, Rumpf LL, Tabrizifard S, Schmitt TM, Zúñiga-Pflücker JC, Petrie HT. Heterogeneity among DN1 prothymocytes reveals multiple progenitors with different capacities to generate T cell and non-T cell lineages. *Immunity*. 2004;20(6):735-745.
40. Shibata K, Yamada H, Nakamura M, et al. IFN- γ -producing and IL-17-producing $\gamma\delta$ T cells differentiate at distinct developmental stages in murine fetal thymus. *J Immunol*. 2014;192(5):2210-2218.
41. Taghon T, Yui MA, Pant R, Diamond RA, Rothenberg EV. Developmental and molecular characterization of emerging beta- and gammadelta-selected pre-T cells in the adult mouse thymus. *Immunity*. 2006;24(1):53-64.
42. Kabashima K, Honda T, Ginhoux F, Egawa G. The immunological anatomy of the skin. *Nat Rev Immunol*. 2019;19(1):19-30.
43. Quresma JAS. Organization of the skin immune system and compartmentalized immune responses in infectious diseases. *Clin Microbiol Rev*. 2019;32(4):e00034-18.
44. Silverberg JI, Kleiman E, Lev-Tov H, et al. Association between obesity and atopic dermatitis in childhood: a case-control study. *J Allergy Clin Immunol*. 2011;127(5):1180-1186.
45. Savetsky IL, Albano NJ, Cuzzone DA, et al. Lymphatic Function Regulates Contact Hypersensitivity Dermatitis in Obesity. *J Invest Dermatol*. 2015;135(11):2742-2752.
46. Sterry W, Strober BE, Menter A, Council IP. Obesity in psoriasis: the metabolic, clinical and therapeutic implications. Report of an interdisciplinary conference and review. *Br J Dermatol*. 2007;157(4):649-655.
47. Miethe S, Guarino M, Alhamdan F, et al. Effects of obesity on asthma: immunometabolic links. *Pol Arch Intern Med*. 2018;128(7-8):469-477.
48. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature*. 1998;394(6696):897-901.
49. Kitajima M, Kimura A, Suzuki H. Cutting edge: Nqo1 regulates irritant contact hypersensitivity against croton oil through maintenance of dendritic epidermal T cells. *J Immunol*. 2018;200(5):1555-1559.
50. Fay NS, Larson EC, Jameson JM. Chronic Inflammation and $\gamma\delta$ T Cells. *Front Immunol*. 2016;7:210.
51. Cheng HY, Wu R, Gebre AK, et al. Increased cholesterol content in gammadelta ($\gamma\delta$) T lymphocytes differentially regulates their activation. *PLoS One*. 2013;8(5):e63746.
52. Schmolka N, Wencker M, Hayday AC, Silva-Santos B. Epigenetic and transcriptional regulation of $\gamma\delta$ T cell differentiation: Programming cells for responses in time and space. *Semin Immunol*. 2015;27(1):19-25.
53. Schmolka N, Serre K, Grosso AR, et al. Epigenetic and transcriptional signatures of stable versus plastic differentiation of proinflammatory $\gamma\delta$ T cell subsets. *Nat Immunol*. 2013;14(10):1093-1100.
54. Takahama Y. Journey through the thymus: stromal guides for T-cell development and selection. *Nat Rev Immunol*. 2006;6(2):127-135.
55. Nakamura M, Shibata K, Hatano S, et al. A genome-wide analysis identifies a notch-RBP-J κ -IL-7R α axis that controls IL-17-producing $\gamma\delta$ T cell homeostasis in mice. *J Immunol*. 2015;194(1):243-251.
56. Michel ML, Pang DJ, Haque SF, Potocnik AJ, Pennington DJ, Hayday AC. Interleukin 7 (IL-7) selectively promotes mouse and human IL-17-producing $\gamma\delta$ cells. *Proc Natl Acad Sci U S A*. 2012;109(43):17549-17554.
57. Shibata K, Yamada H, Sato T, et al. Notch-Hes1 pathway is required for the development of IL-17-producing $\gamma\delta$ T cells. *Blood*. 2011;118(3):586-593.
58. Wencker M, Turchinovich G, Di Marco BR, et al. Innate-like T cells straddle innate and adaptive immunity by altering antigen-receptor responsiveness. *Nat Immunol*. 2014;15(1):80-87.
59. Yang H, Youm YH, Vandanmagsar B, et al. Obesity accelerates thymic aging. *Blood*. 2009;114(18):3803-3812.
60. Howard JK, Lord GM, Matarese G, et al. Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in ob/ob mice. *J Clin Invest*. 1999;104(8):1051-1059.
61. Vasudev A, Ying CT, Ayyadhury S, et al. $\gamma\delta$ T cell subsets in human aging using the classical $\alpha\beta$ T cell model. *J Leukoc Biol*. 2014;96(4):647-655.
62. Prinz I, Sandrock I. Dangerous $\gamma\delta$ T cells in aged mice. *EMBO Rep*. 2019;20(8):e48678.
63. Kimura F, Ito S, Endo Y, et al. Supplementation of DHA-rich microalgal oil or fish oil during the suckling period in mildly n-3 fatty acid-deficient rat pups. *Lipids*. 2011;46(12):1101-1110.
64. Kobayashi S, Phung HT, Tayama S, et al. Fatty acid-binding protein 3 regulates differentiation of IgM-producing plasma cells. *FEBS J*. 2020. Online ahead of print.
65. Cinquina V, Calvigioni D, Farlik M, et al. Life-long epigenetic programming of cortical architecture by maternal 'Western' diet during pregnancy. *Mol Psychiatry*. 2020;25(1):22-36.
66. Harb H, Irvine J, Amarasekera M, et al. The role of PKC ζ in cord blood T-cell maturation towards Th1 cytokine profile and its epigenetic regulation by fish oil. *Biosci Rep*. 2017;37(2):BSR20160485.
67. O'Sullivan D, van der Windt GJ, Huang SC, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity*. 2014;41(1):75-88.

68. Ibitokou SA, Dillon BE, Sinha M, et al. Early Inhibition of Fatty Acid Synthesis Reduces Generation of Memory Precursor Effector T Cells in Chronic Infection. *J Immunol*. 2018;200(2):643-656.
69. van der Windt GJ, Pearce EL. Metabolic switching and fuel choice during T-cell differentiation and memory development. *Immunol Rev*. 2012;249(1):27-42.
70. Monk JM, Turk HF, Fan YY, et al. Antagonizing arachidonic acid-derived eicosanoids reduces inflammatory Th17 and Th1 cell-mediated inflammation and colitis severity. *Mediators Inflamm*. 2014;2014:917149.
71. Lee J, Aoki T, Thumkeo D, Siriwach R, Yao C, Narumiya S. T cell-intrinsic prostaglandin E2-EP2/EP4 signaling is critical in pathogenic TH17 cell-driven inflammation. *J Allergy Clin Immunol*. 2019;143(2):631-643.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Kobayashi S, Phung HT, Kagawa Y, et al. Fatty acid-binding protein 3 controls contact hypersensitivity through regulating skin dermal V γ 4⁺ γ / δ T cell in a murine model. *Allergy*. 2021;76:1776-1788. <https://doi.org/10.1111/all.14630>