Genetic deletion of Cyp4f18 disrupts the omega-3 epoxidation pathway and results in psoriasis-like dermatitis

Mio Yoshida1,2 | Tomoaki Ishihara2 | Yosuke Isobe1,2,3 | Makoto Arita1,2,3

1Division of Physiological Chemistry and Metabolism, Graduate School of Pharmaceutical Sciences, Keio University, Tokyo, Japan
2Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
3Cellular and Molecular Epigenetics Laboratory, Graduate School of Medical Life Science, Yokohama City University, Yokohama, Japan

Correspondence
Makoto Arita, Division of Physiological Chemistry and Metabolism, Graduate School of Pharmaceutical Sciences, Keio University, Minato-ku, Tokyo 105-8512, Japan. Email: arita-mk@pha.keio.ac.jp

Present address
Tomoaki Ishihara, Faculty of Pharmaceutical Sciences, Nagasaki International University, Nagasaki, Japan

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 15H05897, 15H05898 and 20H00495; Exploratory Research for Advanced Technology, Grant/Award Number: JPMJER2101; RIKEN, Grant/Award Number: Glyco-Lipidologue Initiative and Junior Research Associate Program

Abstract
Cyp4f18 catalyzes the conversion of n-3 polyunsaturated fatty acids (PUFAs) into omega-3 epoxides, such as 17,18-epoxyeicosatetraenoic acid (17,18-EpETE) and 19,20-epoxydocosapentaenoic acid (19,20-EpDPE) from eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), respectively. Cyp4f18-deficient mice spontaneously develop psoriasis-like dermatitis. A significant increase in the number of IL-17A-positive gamma delta (γδ) T cells in the skin and enlargement of draining lymph nodes was observed. These symptoms were drastically suppressed by antibiotic treatment. Cyp4f18 is highly expressed in dendritic cells (DCs), and Cyp4f18-deficient bone marrow-derived dendritic cells (BMDCs) show markedly increased expression levels of cytokines such as IL-23 and IL-1β in response to lipopolysaccharide (LPS) stimulation. Lipidomic analysis of lymph nodes and BMDCs revealed a significant decrease in a series of omega-3 epoxidized metabolites. Among them, 17,18-dihydroxyeicosatetraenoic acid (17,18-diHETE), a vicinal diol derived from EPA omega-3 epoxidation suppressed IL-23 production in LPS-stimulated BMDCs in Cyp4f18-deficient mice. These results demonstrate that Cyp4f18 endogenously produces omega-3-epoxidized metabolites in the draining lymph nodes, and these metabolites contribute to skin homeostasis by suppressing the excessive activation of the IL-23/IL-17 axis initiated by DCs.

KEYWORDS
dendritic cell, fatty acid metabolism, lipid mediator, n-3 polyunsaturated fatty acids, psoriasis

Abbreviations: AA, arachidonic acid; BMDC, bone marrow-derived dendritic cell; COX, cyclooxygenase; CYP, cytochrome P450; DC, dendritic cell; DHA, docosahexaenoic acid; diHDoHE, dihydroxydocosahexaenoic acid; diHETE, dihydroxyeicosatetraenoic acid; EPA, eicosapentaenoic acid; EpDPE, epoxydocosapentaenoic acid; EpETE, epoxyeicosatetraenoic acid; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPCR, G protein-coupled receptor; HDiHE, hydroxydocosahexaenoic acid; HETE, hydroxyeicosatetraenoic acid; IL-1β, interleukin-1β; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LOX, lipooxygenase; LPS, lipopolysaccharide; LT, leukotrienes; MAPK, p38 mitogen-activated protein kinase; MaR, maresin; MRM, multiple reaction monitoring; NF-κB, nuclear factor-kappa B; FG, prostaglandins; PPAR, peroxisome proliferator-activated receptor; PUFAs, polyunsaturated fatty acids; Rv, resolvin; sEH, soluble epoxide hydrolase; SPF, specific pathogen-free.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. The FASEB Journal published by Wiley Periodicals LLC on behalf of Federation of American Societies for Experimental Biology.
1 | INTRODUCTION

Fatty acids that contain more than one double bond in the molecule are called polyunsaturated fatty acids (PUFAs), classified as n-6 and n-3 PUFAs based on the position of the double bond from the omega end. For example, n-3 PUFAs are PUFAs with an olefin in the third position from the omega end (i.e., omega-3 double bond). Fatty acid oxygenases, such as cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP), metabolize PUFAs and convert them into various bioactive metabolites. For example, arachidonic acid (AA) is metabolized by COX and LOX to prostaglandins (PG) and leukotrienes (LT), respectively, which initiate the inflammatory response by acting on G protein-coupled receptors (GPCRs). In contrast, eicosapentaenoic acid (EPA)-derived mediators reported to inhibit neutrophil infiltration into inflammatory sites. Among these, Cyp4f18 was characteristically expressed in immune tissues, such as the spleen and lymph nodes (BioGPS: https://www.biogps.org, and Immugen: https://www.immugen.org). This led us to hypothesize that Cyp4f18, possibly through the formation of n-3 PUFA-derived mediators, may regulate inflammation and tissue homeostasis in vivo. Here, we report that Cyp4f18-deficient mice develop spontaneous psoriasis-like dermatitis with excessive activation of the IL-23/IL-17 axis in the regional lymph nodes in a bacteria-dependent manner. Cyp4f18 contributed to the endogenous formation of metabolites derived from not only EPA but also DHA in lymph nodes. We further identified 17,18-dihydroxyeicosatetraenoic acid (17,18-dHETE), a diol generated from an omega-3 epoxide of EPA, as a major bioactive metabolite that inhibits IL-23 and IL-1β production from DCs.

2 | MATERIALS AND METHODS

2.1 | Mouse

Male C57BL/6J mice (aged 4–8 weeks) were purchased from CLEA Japan Inc. (Tokyo, Japan) and bred under specific pathogen-free (SPF) conditions. Cyp4f18 knockout mice on a C57BL/6 background were obtained from the Mutant Mouse Resource and Research Center (MMRRC, UC Davis). Mice between 4 and 24 weeks old were used in all the experiments. All experimental procedures were approved by the Animal Care and Use Committee of RIKEN. The mice were administered 1 mg/ml of ampicillin in drinking water for 1 month for antibiotic treatment.

2.2 | Histological analysis

Hematoxylin and eosin (H&E) staining was performed on the dorsal skin and intestinal tract. Harvested dorsal skin was fixed with 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS; Wako, Osaka, Japan) overnight. Tissues were embedded in paraffin (IEDA TRADING CORPORATION, Tokyo, Japan) with LEICA EG1160 (Leica Biosystems, Nussloch, Germany) to prepare 10-μm sections using a microtome (LEICA RM2145, Leica Biosystems, Nussloch, Germany). Small and large intestines were embedded in the OCT compound (Sakura Finetek Japan Co., Ltd., Tokyo, Japan) and frozen in liquid nitrogen. The sections were stained with hematoxylin and eosin (MUTO PURE CHEMICALS Co., Ltd.,
Tokyo, Japan) and embedded in Entellan™ new (Merck KGaA, Darmstadt, Germany). The histological images were taken by BZ-X710® inverted microscope (Keyence BZ-X710, Keyence Corporation, Osaka, Japan) at 400× magnification.

### 2.3 Quantitative real-time PCR

Dorsal skin and regional lymph nodes were harvested and stored in RNAlater (Thermo Fisher Scientific, Inc. Cleveland, OH, USA) until use. Tissues were homogenized by zirconia beads of 3.0 and 5.0mmφ (TOMY SEIKO CO, LTD, Tokyo, Japan) using Precellys 24 homogenizer (Bertin Technologies, Ile-de-France, France) at a speed of 6000rpm for 15s, twice. RNA from lymph nodes and BMDCs were isolated with an RNeasy mini kit (Qiagen N.V., Venlo, Netherlands) and skin with an RNeasy fibrous tissue mini kit (Qiagen), respectively. The PrimeScript RT Master Mix (Takara Bio Inc., Shiga, Japan) was used for cDNA synthesis. Real-time PCR was performed in a StepOne™ Plus real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) using TB Green® Premix (Takara Bio Inc., Shiga, Japan) using 1640 medium (Wako) and skin with an RNeasy fibrous tissue mini kit (Qiagen), respectively. The relative expression of the target mRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or beta-actin (Actb).

Relative fold changes were calculated using the ΔΔCT method. Target-specific primers used in this study were as follows: IIIβ (Fw: TGCCACCTTTTGACAGTGATG, Rv: AGAAGAACACCACAGAG, Rv: GCAGAGTCTCGCCATTATGATTC), Il23p19 (Fw: CGAGAGTCTCGCCATTATGATTC, Rv: TTCATTGCGGTGGAGAGTCC), S100a8 (Fw: AAATCACCATGCCCTCTACAAG, Rv: CTGTGCCTTGGTAGCATCTATG), and Tslp (Fw: TCGAGGACTGTGAGAG, Rv: ATGCAGAGATTCCGA).

### 2.4 Flow cytometry

Cutaneous cell suspensions were prepared from the shaved dorsal skin. The skin was incubated with 0.25% trypsin in an RPMI EDTA-free medium (Thermo Fisher Scientific) at 37°C for 1 h. Tissues were shredded in an RPMI1640 medium (Wako) containing 0.1 mg/ml Liberase TM (Roche, Basel, Switzerland) and 0.1 mg/ml DNase I (Sigma, St. Louis, MO) and dissociated using the gentle MACS B0.1 program (gentle MACS, Milteny Biotech, North Rhine-Westphalia, Germany).

After the tissues were incubated at 37°C in a thermostatic oscillator, cells were washed with PBS (Thermo Fisher Scientific) containing 2% FCS (Biowest, Nuaille, France) and filtered through 100 μm cell strainers followed by 40 μm cell strainers (CORNING, Corning, NY). Single-cell suspensions were incubated at 37°C for 3 h in a complete RPMI medium (cRPMI) containing RPMI-1640 (Wako) supplemented with 10% fetal bovine serum (FBS) (Biowest), 100 μg/ml streptomycin, 100 U/ml penicillin, 292 μg/ml-glutamine (Gibco), and 55 μM 2-mercaptoethanol (Gibco), 50 ng/ml phorbol 12-myristate 13-acetate (PMA, Wako), and 1 μg/ml ionomycin (Wako) in the presence of 1 μl/1 ml Goldiplug™ Protein Transport Inhibitor (BD Biosciences, Franklin Lakes, NJ). Cells were stained with Ghost Dye™ Red 780 (TONBO biosciences, San Diego, CA, USA) for dead cells and surface cell markers (BioLegend, San Diego, CA). Fixation and permeabilization were performed using IntraPrep Permeabilization Reagent (BECKMAN COULTER, Brea, CA, USA), followed by intracellular IL-17A staining. Cells were acquired on a FACS Aria cell sortor (BD Biosciences) and analyzed using FlowJo software (TreeStar, Ashland, OR, USA). The following antibodies were used for flow cytometry staining: FITC antimouse TCRγ/δ (GL3; BioLegend), PE antimouse IL-17A (TC11-18H10.1; BioLegend), APC antimouse γδ TCR (GL3; BioLegend), PE antimouse CD4 (12A; BioLegend), and BV421 antimouse CD4 (GK1.5; BioLegend). All antibodies were used at 1:250 dilutions.

#### 2.5 Targeted liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based lipidomics

LC–MS/MS-based targeted lipidomics was performed as previously described. Briefly, frozen regional lymph nodes in ice-cold methanol (Wako) were homogenized by Zirconia beads of 3.0 and 5.0mmφ (TOMY) using a Precellys 24 homogenizer (Bertin Technologies) and kept at −30°C overnight. The methanolic supernatant was applied to MonoSpin C18-AX cartridges (GL Science, Tokyo, Japan) to extract lipid metabolites in the presence of deuterated internal standards: 1 ng of arachidonic acid (AA)-d8, 15-hydroxyeicosatetraenoic acid (HETE)-d8, LTB4-d4, LTD4-d5, and prostaglandin E2 (PGE2)-d4 (Cayman Chemical, Ann Arbor, MI) for monitoring recovery rates during sample preparation. For LC–MS/MS analysis, a triple-quadrupole linear ion-trap mass spectrometer (4500QTRAP, AB Sciex, Foster City, CA) equipped with an ACQUITY UPLC BEH C18
column (1.0 × 150 mm, 1.7-μm particle size; Waters Corp., Milford, MA) was used. MS/MS analysis was performed in the negative ion mode, and metabolites were identified and quantified by multiple reaction monitoring (MRM). Calibration curves between 1 and 1000 pg and the LC retention times for each compound were established using synthetic standards. Raw data were analyzed using MultiQuant™ software (Sciex).

2.6 | Bone marrow-derived dendritic cells

Bone marrow cells were flushed out from the thigh- and shinbones of mice and lysed with Red Blood Cell Lysing Buffer Hybri-Max (Sigma-Aldrich). The cells were suspended in cRPMI, and 2 × 10^6 cells were seeded in a 10 cm dish in 20 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF; Peprotech, Cranbury, NJ, USA). After 3 days of incubation at 37°C and 5% CO₂, 10 ml of cRPMI containing GM-CSF was added and incubated for another 3 days. Ten milliliters of the supernatant were changed to fresh medium and incubated for 2 days, and the cells on day 9 were used for the experiment. For RT-qPCR analysis, sorted PI − CD45^+ CD11c^+ F4/80^- cells (BMDCs) (FACSAria, BD Biosciences) were adjusted to 1 × 10^6 cells/ml and seeded onto 12-well plates. LPS from Escherichia coli. O111:B4 (Sigma-Aldrich) was added, and the cells were incubated for 24 h. BMDCs were collected with Buffer RLT (RNeasy Mini Kit, Qiagen) and fractured using a 27G syringe. The samples were stored at −80°C until use. The following antibodies were used for flow cytometry staining: FITC antimouse F4/80 (BM8; BioLegend; 1:250 dilution), APC antimouse CD45 (104; BioLegend; 1:250 dilution), PE antimouse CD11c (N418; BioLegend; 1:250 dilution), BV421 antimouse MHC II (M5/114.15.2; BioLegend; 1:250 dilution), and propidium iodide (PI) solution (Sigma-Aldrich; 1:1000 dilution).

2.7 | Enzyme-linked immunosorbent assay (ELISA)

BMDCs were prepared at 8 × 10^5 cells/ml and seeded into 96-well plates and stimulated with 1000 ng/ml LPS (Sigma-Aldrich) 30 min after the addition of fatty acid metabolites (17,18-EpETE, 17,18-diHETE, 19,20-EpDPE, 19,20-diHDoPE, 5,6-diHETE, 8,9-diHETE, 11,12-diHETE, and 14,15-diHETE; Cayman Chemicals). After 24 h, the culture supernatant was collected and subjected to ELISA. The following kits were used for cytokine measurement by ELISA: ELISA MAX™ DELUXE Set Mouse IL-1β, and ELISA MAX™ DELUXE Set Mouse IL-6 (BioLegend). The experiments were performed according to the manufacturer’s instructions.

2.8 | Western blotting

Frozen dorsal skin and lymph nodes in RIPA buffer (Thermo Fisher Scientific) with cOmplete™ mini EDTA-free protease inhibitor cocktail (Roche) were homogenized by Zirconia beads of 3.0 and 5.0 mmφ (TOMY) using a Precellys 24 homogenizer (Bertin Technologies). Protein quantification was performed using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). Proteins were separated by SDS-PAGE and transferred to Immune-Blot PVDF Membranes for Protein Blotting (BioRad, Hercules, CA, USA) using the Trans-Blot Turbo™ Transfer System (BioRad). Blots were blocked with EveryBlot blocking Buffer (BioRad) for 5 min at RT and probed with primary antibody overnight at 4°C. Blots were washed three times with Tris-buffered saline (TBS) containing Tween 20 (TBS-T) solution and then reacted with the secondary antibody for 1 h at room temperature. Blots were visualized using ChemiDoc touch (BioRad). An anti-Cyp4f18 polyclonal antibody was prepared from a rabbit by immunization with a synthetic peptide of mouse Cyp4f18 (LPDDKEPRRKPEL; Eurofin Genomics, Tokyo, Japan). Anti-Gapdh mouse monoclonal antibody was purchased from Sigma-Aldrich.

2.9 | RNA sequence

RNA extraction of lymph nodes and dendritic cells was performed as described above. After confirming RINs with Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), libraries were prepared using SureSelect Strand-Specific RNA library Prep System (Agilent Technologies). Measurements were performed using Hiseq1500 (Illumina, San Diego, CA, USA). Data mapping was performed using Tophat, and fpkm was calculated using cufflinks.

2.10 | Statistical analysis

Results are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using Excel and R software. Two-tailed Student’s t test was used to compare the difference between the two groups. Dunnett’s test and Tukey’s honestly significant difference (HSD) were used to compare the differences among multiple groups. Statistical significance was set at p < .05.
3 | RESULTS

3.1 | Cyp4f18 KO mice developed spontaneous psoriasis-like dermatitis

Phenotype analysis of Cyp4f18 KO mice revealed the spontaneous disruption of skin homeostasis. Histological images showed thickening of the epidermis and dermis and infiltration of inflammatory cells into the subcutaneous tissue (Figure 1A). Notably, this phenotype became prominent in Cyp4f18 KO mice aged 16 weeks. We then analyzed the gene expression of skin disease markers in dorsal skin. We observed that psoriasis markers (i.e., S100a8 and S100a9) were markedly upregulated in Cyp4f18 KO mice. In contrast, the expression of Tslp, a marker of allergic dermatitis, was not different from that in WT mice (Figure 1B). IL-17 is reportedly involved in the pathogenesis of psoriasis. Therefore, we compared Il17a expression using RT-qPCR and the number of IL-17-producing cells in the dorsal skin using flow cytometry. Il17a expression was upregulated in the dorsal skin of Cyp4f18 KO mice compared with that in WT mice. The number of IL-17A+ gamma delta (γδ) T cells was significantly increased in Cyp4f18 KO mice, whereas no significant difference was observed in the number of Th17 cells (Figure 1C–E, Figure S1). Because the gastrointestinal tract is in contact with the outside world like skin, it is constantly at risk of infection and there is a constitutive accumulation of a variety of immune cells, including Th17 cells. Therefore, we observed the histology of the intestinal tracts to confirm whether inflammation occurs in those tissues. However, no inflammation was observed in Cyp4f18 KO mice. (Figure S2) These results indicated that Cyp4f18 KO mice spontaneously developed psoriasis-like dermatitis with local activation of IL-17A+ γδ T cells in the skin.
3.2 | Enlargement and enhanced activation of IL-23/IL-17 axis in the skin-draining lymph nodes of Cyp4f18 KO mice

Protein expression of Cyp4f18 was observed in lymph nodes but not in the skin (Figure S3A). Cyp4f18 is expressed in immune cells, including DCs, B cells, and neutrophils (http://rstats.immgen.org/Skyline_microarray/skyline.html, Figure S3B). We focused on skin-draining lymph nodes based on this expression pattern, where antigen-stimulated DCs activate T cells to produce IL-17. As shown in Figure 2A, skin-draining lymph nodes were enlarged in Cyp4f18 KO mice than those in WT mice. Lymph nodes at other sites did not differ in size between WT and Cyp4f18 KO (Figure S4). This enlargement was observed in Cyp4f18 KO mice at 16 weeks of age or more (data not shown). In psoriasis, activated DCs produce excessive IL-23 and IL-1β to promote IL-17 production by γδ T cells. To examine whether the IL-23/IL-17 axis was activated in regional lymph nodes, RT-qPCR analysis was performed. As expected, Il1b, Il17a, and Il23p19 were upregulated in the regional lymph nodes of Cyp4f18 KO mice, whereas the expression of Th1- and Th2-related cytokines was unchanged (Figure 2C). As observed in the skin, the number of IL-17+ γδ T cells also increased in the lymph nodes of Cyp4f18 KO mice (Figure 2D). These results demonstrated that the IL-23/IL-17 pathway is excessively activated in the skin-draining lymph nodes of Cyp4f18 KO mice.

3.3 | Spontaneous dermatitis in Cyp4f18KO mice was bacteria dependent

Altered skin flora has been reported in psoriasis lesions, and studies using germ-free (GF) mice demonstrated that IL-17 production in the skin was bacteria dependent. To determine whether the same held true for skin inflammation observed in Cyp4f18 KO mice, we administered ampicillin (1 mg/ml) to 12-week-old mice for 1 month through drinking water. Although thickening of the skin and infiltration of inflammatory cells were observed in Cyp4f18 KO mice drinking distilled water (control), these symptoms were significantly suppressed in the antibiotic-consuming group (Figure 3A). Enhanced gene expression of psoriasis markers in the dorsal skin of Cyp4f18 KO mice was entirely suppressed by antibiotics to the same extent as in WT (Figure 3B–D). The enlargement of the regional lymph nodes observed in Cyp4f18 KO mice was also significantly suppressed by antibiotic treatment (Figure 3E). Antibiotic-mediated inhibition of the activation of the IL-23/IL-17 axis in skin-draining lymph nodes of Cyp4f18 KO mice corroborated the finding mentioned earlier (Figure 3F–H). These results indicate that the skin inflammation observed in Cyp4f18 KO mice was bacteria dependent.

3.4 | Dendritic cells derived from Cyp4f18 KO mice exhibited an excessive cytokine response to lipopolysaccharide (LPS) stimulation

Bacterial stimuli in the skin are recognized via pattern recognition receptors on DCs, which stimulate the production of inflammatory cytokines. In psoriasis pathogenesis, IL-23 and IL-1β produced by DCs are essential for maintaining the function of IL-17-producing T cells. In addition, the expression of Cyp4f18 was higher in DCs than in γδ T cells and CD4+ T cells in the spleen (Figure 4A), leading us to focus our analysis on DCs. Bone marrow-derived dendritic cells (BMDCs) from WT and Cyp4f18 KO mice were stimulated with LPS for 24 hours, and the expression of IL-23 and IL-1β was evaluated. The results showed that BMDCs derived from Cyp4f18 KO mice exhibited excessive cytokine induction in response to LPS stimulation (Figure 4B,C). This cytokine hyper-response was not only observed with IL-23 and IL-1β, which are closely related to psoriasis but also with other cytokines such as TNFα and IL-6 (Figure 4D–F). Collectively, these results indicated that BMDCs from Cyp4f18 KO mice showed an excessive response to LPS stimulation.

3.5 | Impaired n-3 PUFA metabolism in the lymph nodes of Cyp4f18 KO mice

Cyp4f18 functions as a fatty acid oxygenase that catalyzes the oxygenation of omega-1 and omega-2 and converts LTB4 into 19-OH-LTB4 and 18-OH-LTB4, and EPA into 19-hydroxyeicosapentaenoic acid (19-HEPE). We previously identified Cyp4f18 as one of the enzymes responsible for omega-3 epoxidation of EPA to produce 17,18-EpETE. The other four enzymes producing 17,18-EpETE were barely expressed in lymph nodes and dendritic cells, and there was no change in their expression levels due to the loss of Cyp4f18 (Figure S3B,C). Here, we performed LC–MS/MS-based mediator lipidomics to determine whether Cyp4f18 is responsible for the endogenous production of these PUFA metabolites in vivo. As shown in Figure 5, Cyp4f18-dependent production of a series of n-3 PUFA metabolites, such as 19-HEPE, 21-hydroxydocosahexaenoic acid (21-HDoHE), 17,18-diHETE, 19,20-epoxydocosapentaenoic acid (19,20-EpDPE), and 19,20-dihydroxydocosahexaenoic acid...
(19,20-diHDoHE), was observed in the skin-draining lymph nodes. Cyp4f18-derived n-6 PUFA metabolites such as 19-OH-LTB4 and 18-OH-LTB4 were below the detection limit (data not shown). These results demonstrate that Cyp4f18 is responsible for the endogenous production of various n-3 PUFA metabolites in vivo.

3.6  17,18-diHETE inhibited IL-23 production from DCs

Similar metabolic changes in BMDCs and lymph nodes (Figure S5) suggested that the metabolites produced via Cyp4f18 in BMDCs may contribute to this phenotype. Mouse BMDCs were treated with n-3 PUFA metabolites, including 17,18-EpETE, 17,18-diHETE, 19,20-EpDPE, and 19,20-diHDoHE, and IL-23 production, in response to LPS were monitored. 17,18-diHETE exerted potent inhibitory effects on DC activation, whereas 17,18-EpETE and 19,20-EpDPE were less potent, and 19,20-diHDoPE was inactive (Figure 6A). Administration of 17,18-diHETE also suppressed the production of IL-1β mRNA in response to LPS, whereas IL-6 and TNFα mRNA levels were unaffected (Figure 6B). IL-1β upregulation and its suppression by 17,18-diHETE in Cyp4f18-deficient BMDCs was observed also at the protein levels (Figure S6). We next evaluated a series of EPA metabolites with different diol positions (i.e., 5,6-diHETE, 8,9-diHETE, 11,12-diHETE, and 14,15-diHETE) to determine the structural selectivity of 17,18-diHETE, which revealed that only 17,18-diHETE inhibited IL-23 production from LPS-stimulated BMDCs (Figure 6C). While the molecular target of 17,18-diHETE has not yet been identified, 17,18-EpETE, a precursor of 17,18-diHETE, reportedly has a direct agonist activity against GPR40 and GPR120 and exerts anti-inflammatory effects via PPARγ.13,16 Therefore, we treated BMDC with 17,18-diHETE in the presence of chemical inhibitors to assess whether the effects of 17,18-diHETE are mediated through these receptors. The results collectively demonstrate that the inhibitory effect of 17,18-diHETE on IL-23 production was not suppressed by any of these inhibitors, suggesting that the action of 17,18-diHETE on BMDCs is mediated through different targets (Figure S7).
DISCUSSION

In this study, we demonstrated the importance of the Cyp4f18-mediated omega-3 epoxidation pathway in maintaining skin homeostasis. In addition, we identified 17,18-diHETE as a novel bioactive metabolite that counterregulates pro-inflammatory signals in DCs.

Psoriasis is a chronic skin disease characterized by erythematous plaques with scaling, affecting approximately 0.3% of the population in Japan and 3% in the West.29,30
Psoriasis is caused by various environmental factors, such as diet, stress, and genetic predisposition; however, the pathogenic mechanism underlying such functions is not fully understood. In the pathogenesis of psoriasis, IL-23 and IL-1β produced by DCs, and subsequent activation of IL-17-producing cells are essential. In mice lacking Langerhans cells, imiquimod (IMQ)-induced psoriasis is suppressed due to the decreased number of γδ T cells and related cytokines in the epidermal tissue-resident DC population. In addition, plasmacytoid DC (pDC) contributes to the induction of psoriasis, with inflammatory DCs (iDC) being major players in psoriatic inflammation, suggesting the involvement of various subsets of DCs in psoriasis pathogenesis. IL-23, with IL-1β, enhances IL-17 production by T cells. The dramatic suppression of IMQ-induced psoriasis inflammation in mice lacking IL-23 or IL-17 receptors and the use of anti-IL-23 and anti-IL-17 antibodies as effective treatments in patients with psoriasis indicates that the IL-23/IL-17 axis is an essential cytokine pathway in the pathogenesis of psoriasis. We identified 17,18-diHETE as a novel bioactive metabolite that counterregulates IL-1β and IL-23 production in LPS-activated DCs. Cyp4f18 deletion decreased the amount of 17,18-diHETE in immune tissues and caused spontaneous psoriasis-like dermatitis. Although the detailed mechanisms require further investigation, these findings suggest that dermatitis resulting from the deletion of Cyp4f18 may be due to an increase in psoriasis-related cytokines, such as IL-1β and IL-23, from DCs, possibly through the dysregulation of Cyp4f18-derived functional metabolites, including 17,18-diHETE.

The spontaneous onset of psoriatic dermatitis in Cyp4f18 KO mice was significantly suppressed upon antibiotic treatment. These results indicate that skin inflammation is triggered by bacterial stimuli. A strong relationship between commensal skin bacteria and inflammation has been suggested in patients with psoriasis. It has been documented that Th17-related inflammation is induced by S. aureus infection. Based on these, we speculate that the presence of staphylococci may be involved in the onset and/or progression of dermatitis in Cyp4f18 KO mice. In addition, dysbiosis of the skin tissue was observed in the inflamed area of psoriasis, with an increase in Streptococcus and a decrease in

![Graphs and images illustrating the results of experiments on Cyp4f18 KO-derived BMDCs.](image-url)
Analysis of bacterial flora on the skin of Cyp4f18 KO mice may help identify commensal bacteria responsible for dermatitis. n-3 PUFA metabolites reportedly improve the symptoms in a mouse model of psoriasis. For example, in the IL-23 subcutaneous injection-induced psoriasis model, maresin-1 (MaR1) suppressed skin hyperplasia and infiltration of inflammatory cells by attenuating IL-23 receptor expression on Th17 and γδ T cells by inhibiting RORγt and IL-17A production from T cells.40 In the IMQ-induced psoriasis model, Fat-1 transgenic mice endogenously produced n-3 PUFAs, and mice treated with RvE1 or RvD1 showed improved inflammation via suppressing the IL-23/IL-17 axis.41–43 Although a series of n-3 fatty acid-derived bioactive metabolites are antipsoriatic, they have been examined by exogenous administration. The role of endogenous fatty acid metabolism in psoriasis development is not well characterized. This study suggests that endogenous 17,18-diHETE produced via Cyp4f18 acts on DCs in an autocrine and/or paracrine manner, contributing to skin homeostasis. Also, our study demonstrates that an EPA-rather than DHA-enriched diet may be more efficient from a nutritional perspective in the prevention and/or treatment of psoriasis. Future elucidation of the target of 17,18-diHETE in DCs will lead to evidence-based nutritional approaches for the treatment and/or prevention of psoriasis.

Epoxy fatty acids, including 17,18-EpETE and 19,20-EpDPE, are rapidly metabolized into vicinal diols by soluble epoxide hydrolase (sEH), 44 and 17,18-diHETE has been described as an inactive form of 17,18-EpETE in previous studies.13,16 Also, the diol of linoleate has been reported to show a negative biological effect than the epoxide at high concentrations.45 However, some fatty acid diols have recently been shown to exhibit bioactivity. For example, vicinal diols derived from linoleic acid have been reported to promote fatty acid uptake in adipose tissue and skeletal muscle and reduce the abundance of Treg cells.46–48 Here, we observed a unique and novel finding that 17,18-diHETE is bioactive in the regulation of DCs in an autocrine and/or paracrine manner.
activation. Cytokine suppression in DCs by 17,18-diHETE was not inhibited in GPR40, GPR120, and PPARγ inhibitors (Figure S5), suggesting the presence of an unknown target. Deletion of Cyp4f18 also decreases 17,18-EpETE, which inhibits the nuclear factor-κB (NF-κB) signaling pathway, implying that the combination of Cyp4f18-dependent n-3 PUFA metabolites exerts counterregulatory signals to maintain skin homeostasis.

In conclusion, this study demonstrated the first tissue-protective effects of Cyp4f18 that promotes the omega-3 epoxidation pathway of n-3 PUFAs in vivo. These findings collectively illustrate the involvement of endogenous n-3 fatty acid metabolism in the regulation of inflammation and suggest the therapeutic potential of n-3 fatty acid-derived bioactive metabolites in dermatitis and other inflammatory diseases. Further investigations on Cyp4f18- and Cyp4f18-derived metabolites will help uncover the physiological significance of the endogenous omega-3 epoxidation pathway of n-3 PUFAs in controlling health and diseases.
AUTHOR CONTRIBUTIONS
Mio Yoshida conducted the experiments, analyzed the results, and wrote the manuscript. Tomoaki Ishihara conducted the experiments and analyzed the results. Yosuke Isobe analyzed the results and wrote the manuscript. Makoto Arita designed the study, analyzed the results, and wrote the manuscript. All authors have reviewed the results and approved the final version of the manuscript.

ACKNOWLEDGMENTS
This work was supported by the JSPS KAKENHI 15H05897, 15H05898, 20H00495 (M.A.), RIKEN Pioneering Project “Glyco-Lipidologue Initiative” (M.A.), JST-ERATO “ARITA Lipidome Atlas Project” grant number JPMJER2101 (M.A.), RIKEN Junior Research Associate Program, and the Keio University Doctorate Student Grant-in-Aid Program from the Ushioda Memorial Fund (M.Y.).

DISCLOSURES
The authors declare that they have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT
Included in the article.

ORCID
Mio Yoshida https://orcid.org/0000-0002-8564-9356

REFERENCES
1. Schmitz G, Ecker J. The opposing effects of n-3 and n-6 fatty acids. Prog Lipid Res. 2008;47:147-155.
2. Hata AN, Breyer RM. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. Pharmacol Ther. 2004;103:147-166.
3. Yokomizo T, Nakamura M, Shimizu T. Leukotriene receptors as potential therapeutic targets. J Clin Invest. 2018;128:2691-2701.
4. Dyerberg J, Bang HO, Hjorne N. Fatty acid composition of the plasma lipids in Greenland Eskimos. Am J Clin Nutr. 1975;28:958-966.
5. Kang JX, Wang J, Wu L, Kang ZB. Transgenic mice: fat-1 mice convert n-6 to n-3 fatty acids. Nature. 2004;427:504.
6. Ninomiya T, Nagata M, Hata J, et al. Association between ratio to disclose. Risk of serum eicosapentaenoic acid to arachidonic acid and risk of cardiovascular disease: the Hisayama study. Atherosclerosis. 2013;231:261-267.
7. Isobe Y, Arita M, Matsueda S, et al. Identification and structure determination of novel anti-inflammatory mediator resolvin E3, 17,18-dihydroxyeicosapentaenoic acid. J Biol Chem. 2012;287:10525-10534.
8. Tjonahen E, Oh SF, Siegelman J, et al. Resolvin E2: identification and anti-inflammatory actions: pivotal role of human 5-lipoxygenase in resolvin E series biosynthesis. Chem Biol. 2006;13:1193-1202.
9. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 selectively interacts with leukotriene B4 receptor BLT1 and ChemR23 to regulate inflammation. J Immunol. 2007;178:3912-3917.
10. Hecker M, Sommer N, Foch S, et al. Resolvin E1 and its precursor 18R-HEPE restore mitochondrial function in inflammation. Biochim Biophys Acta Mol Cell Biol Lipids. 2018;1863:1016-1028.
11. Li J, Chen CY, Arita M, et al. An omega-3 polysaturated fatty acid derivative, 18-HEPE, protects against CXCR4-associated melanoma metastasis. Carcinogenesis. 2018;39:1380-1388.
12. Endo J, Sano M, Isobe Y, et al. 18-HEPE, an n-3 fatty acid metabolite released by macrophages, prevents pressure overload-induced maladaptive cardiac remodeling. J Exp Med. 2014;211:1673-1687.
13. Morin C, Sirois M, Echave V, Albadine R, Rousseau E. 17,18-epoxyeicosatetraenoic acid targets PPARgamma and p38 mitogen-activated protein kinase to mediate its anti-inflammatory effects in the lung: role of soluble epoxide hydrolase. Am J Respir Cell Mol Biol. 2010;43:564-575.
14. Morin C, Sirois M, Echave V, Rizzallah E, Rousseau E. Relaxing effects of 17(18)-EpETE on arterial and airway smooth muscles in human lung. Am J Physiol Lung Cell Mol Physiol. 2009;296:L130-L139.
15. Kunisawa J, Arita M, Hayasaka T, et al. Dietary omega3 fatty acid exerts anti-allergic effect through the conversion to 17,18-epoxyeicosatetraenoic acid in the gut. Sci Rep. 2015;5:9750.
16. Nagatake T, Shiogama Y, Inoue A, et al. The 17,18-epoxyeicosatetraenoic acid-G protein-coupled receptor 40 axis ameliorates contact hypersensitivity by inhibiting neutrophil mobility in mice and cromolyn macaques. J Allergy Clin Immunol. 2018;142:470-484.e12.
17. Mochimaru T, Fukunaga K, Miyata J, et al. 12-OH-17,18-Epoxyeicosatetraenoic acid alleviates eosinophilic airway inflammation in murine lungs. Allergy. 2018;73:369-378.
18. Kubota T, Arita M, Isobe Y, et al. Eicosapentaenoic acid is converted via omega-3 epoxygenation to the anti-inflammatory metabolite 12-hydroxy-17,18-epoxyeicosatetraenoic acid. FASEB J. 2014;28:586-593.
19. Isobe Y, Itagaki M, Ito Y, et al. Comprehensive analysis of the mouse cytochrome P450 family responsible for omega-3 epoxidation of eicosapentaenoic acid. Sci Rep. 2018;8:7954.
20. Arita M. Mediator lipidomics in acute inflammation and resolution. J Biochem. 2012;152:313-319.
21. Cai Y, Shen X, Ding C, et al. Pivotal role of dermal IL-17-producing gammadelta T cells in skin inflammation. Immunity. 2011;35:596-610.
22. Nakajima K, Kanda T, Takaishi M, et al. Distinct roles of IL-23 and IL-17 in the development of psoriasis-like lesions in a mouse model. J Immunol. 2011;186:4481-4489.
23. Singh TP, Schon MP, Wallbrecht K, et al. 8-methoxypsoralen plus ultraviolet a therapy acts via inhibition of the IL-23/Th17 axis and induction of Foxp3 regulatory T cells involving CTLA4 signaling in a psoriasis-like skin disorder. J Immunol. 2010;184:7257-7267.
24. Lowes MA, Kikuchi T, Fuentes-Duculan J, et al. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol. 2008;128:1207-1211.
25. Res PC, Piskin G, de Boer OJ, et al. Overrepresentation of IL-17A and IL-22 producing CD8 T cells in lesional skin suggests
their involvement in the pathogenesis of psoriasis. *PLoS One*. 2010;5:e14108.

26. Nakamizo S, Egawa G, Honda T, Nakajima S, Belkaid Y, Kabashima K. Commensal bacteria and cutaneous immunity. *Semin Immunopathol*. 2015;37:73-80.

27. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity*. 2009;31:331-341.

28. Christmas P, Tolentino K, Primo V, et al. Cytochrome P-450 4F18 is the leukotriene B4 omega-1/omega-2 hydroxylase in mouse polymorphonuclear leukocytes: identification as the functional orthologue of human polymorphonuclear leukocyte CYP4F3A in the down-regulation of responses to LTB4. *J Biol Chem*. 2006;281:7189-7196.

29. Goto H, Nakatani E, Yagi H, Moriki M, Sano Y, Miyachi Y. Late-onset development of psoriasis in Japan: a population-based cohort study. *JAAD Int*. 2021;2:51-61.

30. Zeng J, Luo S, Huang Y, Lu Q. Critical role of environmental factors in the pathogenesis of psoriasis. *Curr Drug Targets Inflamm Allergy*. 2004;3:121-128.

31. Xiao C, Zhu Z, Sun S, et al. Activation of Langerhans cells promotes the inflammation in imiquimod-induced psoriasis-like dermatitis. *J Dermatol Sci*. 2017;85:170-177.

32. Yoshiki R, Kabashima K, Honda T, et al. IL-23 from Langerhans cells is required for the development of imiquimod-induced psoriasis-like dermatitis by induction of IL-17A-producing gammadelta T cells. *J Invest Dermatol*. 2014;134:1912-1921.

33. Glitnzer E, Korosec A, Brunner PM, et al. Specific roles for dendritic cell subsets during initiation and progression of psoriasis. *EMBO Mol Med*. 2014;6:1312-1327.

34. Singh TP, Zhang HH, Borek I, et al. Monocyte-derived inflammatory Langerhans cells and dermal dendritic cells mediate psoriasis-like inflammation. *Nat Commun*. 2016;7:13581.

35. Ito Y, Sasaki T, Li Y, et al. Staphylococcus cohnii is a potentially biotherapeutic skin commensal alleviating skin inflammation. *Cell Rep*. 2021;35:109052.

36. Naik S, Bouladoux N, Wilhelm C, et al. Compartmentalized control of skin immunity by resident commensals. *Science*. 2012;337:1115-1119.

37. Yan D, Issa N, Afifi L, Jeon C, Chang HW, Liao W. The role of the skin and gut microbiome in psoriatic disease. *Curr Dermatol Rep*. 2017;6:94-103.

38. Qin S, Wen J, Bai XC, et al. Endogenous n-3 polyunsaturated fatty acids protect against imiquimod-induced psoriasis-like inflammation via the IL-17/IL-23 axis. *Mol Med Rep*. 2014;9:2097-2104.

39. Sawada Y, Honda T, Nakamizo S, et al. Resolvin E1 attenuates murine psoriatic dermatitis. *Sci Rep*. 2018;8:11873.

40. Xu J, Duan X, Hu F, et al. Resolvin D1 attenuates imiquimod-induced mice psoriasiform dermatitis through MAPKs and NF-kappaB pathways. *J Dermatol Sci*. 2018;89:127-135.

41. Zhang G, Kodani S, Hammock BD. Stabilized epoxygenated fatty acids regulate inflammation, pain, angiogenesis and cancer. *Prog Lipid Res*. 2014;53:108-123.

42. Moghaddam MF, Grant DF, Cheek JM, Greene JF, Williamson KC, Hammock BD. Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat Med*. 1997;3:562-566.

43. Lynes MD, Leiria LO, Lundh M, et al. The cold-induced lipokine 12,13-diHOME promotes fatty acid transport into brown adipose tissue. *Nat Med*. 2017;23:631-637.

44. Stanford KI, Lynes MD, Takahashi H, et al. 12,13-diHOME: an exercise-induced Lipokine that increases skeletal muscle fatty acid uptake. *Cell Metab*. 2018;27:1111-1120.e3.

**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Yoshida M, Ishihara T, Isebo Y, Arita M. Genetic deletion of Cyp4f18 disrupts the omega-3 epoxidation pathway and results in psoriasis-like dermatitis. *The FASEB Journal*. 2022;36:e22648. doi:10.1096/fj.202200982R